JOURNAL OF AGRICULTURAL RESEARCH

VOL. 71 WASHINGTON, D. C., NOVEMBER 15, 1945 No. 10

CYTOPLASMICALLY INHERITED MALE-STERILITY IN SUGAR BEETS [1]

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INTRODUCTION

Male-sterility, which appears to be relatively common in beets (*Beta vulgaris* L.), may have different modes of inheritance. The present paper deals chiefly with male-sterility as inherited in cross-pollinated varieties of sugar beets bred for resistance to curly top (*1*).[2]

To explain the transmission of the male-sterile tendency from parent to offspring in these beets it is necessary to consider a cytoplasmic basis of inheritance (*12*). Literature reviews by Correns (*3*) and Sirks (*15*) reveal the interesting fact that male-sterility in cross-pollinated plants appears to be more commonly explained by cytoplasmic inheritance than by genic, or Mendelian, inheritance. According to mathematical calculations by Lewis (*6*), there may be an opportunity for survival of male-sterile plants in cross-pollinated populations if the male-sterility is inherited cytoplasmically, but the opportunity for survival is very poor if the inheritance is through either dominant or recessive Mendelian factors. Mendelian factors responsible for male-sterility have been frequently encountered in artificially inbred populations of several species of plants (*6*), but such factors do not appear to operate to any marked extent in open-pollinated populations.

In connection with cytoplasmic inheritance, several instances of genic effects that modify the cytoplasmic effects have been reported (*6, 7, 15*). In flax (*2, 4*), a single gene apparently determines male-sterility with one type of cytoplasm but not with another. The inheritance of male-sterility in beets appears to be similar to that described in flax, although the evidence indicates the operation of at least two and possibly more Mendelian factors in beets. The operation of the Mendelian factors in beets also gives rise to semi-male-sterile types.

MATERIAL AND METHODS

In the investigations reported here, cytoplasmically inherited male-sterility was found in varieties and strains derived directly or indirectly from the sugar-beet variety U. S. 1 (*1*), which was the first of the curly-top-resistant sugar-beet varieties released by the United States Department of Agriculture. About 2 percent of the plants in this variety were observed to be male-sterile but female-fertile. Because

[1] Received for publication January 31, 1944.

[2] Italic numbers in parentheses refer to Literature Cited, p. 440.

of self-sterility, cross-pollination prevailed as in most commercial varieties of sugar beets. Mass selection was the principal method of improvement, selections being based largely on disease resistance, size of beet, and sugar content. Some of the mass selections from U. S. 1 showed no male-sterility, whereas others showed an increased percentage. About 6 percent of the beets were observed to be male-sterile in the variety U. S. 33, derived from seed produced from 100 beets selected from U. S. 1. Some strains derived by mass selection from U. S. 33 showed a further increased percentage of male-sterile beets.

The Munerati annual beet [3] and the curly-top-resistant strain 12c (*11*), which was not derived from the U. S. 1 variety, were used in the investigation of male-sterility not cytoplasmically inherited.

Intensive studies of breeding behavior were made possible by maintaining some of the parental beets for a period of years by asexual propagation (*10*).

Appropriate crosses were made by exchanging paper bags over the inflorescences in the greenhouse. Experience showed that contamination from foreign pollen in the greenhouse was extremely rare. Except for limited use of self-fertile types (*11*), most material was so strongly self-sterile that emasculation was deemed unnecessary in connection with hybridization work, regardless of whether the beets were also male-sterile. The completeness of the self-sterility was checked by observation through transparent Pyralin windows in the paper bags. In several instances the transmission of the *R* (*13*) color factor furnished an additional check on the completeness of the self-sterility. Nonemasculated *r r* beets fertilized with pollen from homozygous *R R* beets produced offspring all of which carried the *R* factor, showing that no selfing had taken place.

A limited number of crosses were made by open pollination in the field in connection with breeding work.

Populations for classification were grown either in the greenhouse or in the field, according to convenience and amount of seed available. To prevent contamination from old ungerminated seed, the greenhouse soil was always steam-sterilized before seed was planted. In the field, an occasional error was possible because of contamination by ungerminated beet seed from a previous beet-seed crop, which had remained in the soil for 5 or 6 years. This source of contamination was too small to affect the general conclusions, but it may explain occasional instances of nonconformity.

Environmental variability made classification work difficult. The variable nature of the semi-male-sterile types caused the most difficulty. Usually it was necessary to pull the beets as the classifications were made, although more critical classifications were made in a few instances by periodic observations of beets held in greenhouse pots for a period of about 3 weeks during flowering. Conflicting classifications of data obtained from observations made on different dates, and a limited amount of variability among the different flowers of a plant on any given date, showed that classifications for degree of semi-male-sterility were subject to considerable error. Strong degrees of semi-male-sterility were sometimes confused with complete male-sterility, and beets with lesser degrees of semi-male-sterility were not infrequently classified as apparently normal.

[3] Obtained from Dr. O. Munerati, Director, R. Stazione Sperimentale di Bieticultura, Rovigo, Italy.

THEORETICAL ASSUMPTIONS

Although several Mendelian factors may influence the degree of male-sterility and there may be some instability or variation of substances carried by the cytoplasm, most of the data here recorded may be explained by two Mendelian factors carried by beets having two kinds of cytoplasm.

Assuming two types of cytoplasm, namely, that carried by normal hermaphrodites and that carried by male-sterile beets, they will be indicated by the symbols N and S, respectively. It will be assumed that semi-male-sterile types also carry S cytoplasm but that their appearance is modified by two Mendelian factors, *X* and *Z*. Completely male-sterile beets will be assumed to be of constitution S *x x z z*. The possible constitutions of heterozygous semi-male-sterile beets are S *X x z z*, S *x x Z z*, and S *X x Z z*. There may be a difference between effects produced by the factors *X* and *Z*, but since there is no evidence for such a difference, semi-male-sterile beets of constitution S *X x z z* will be considered indistinguishable from beets of constitution S *x x Z z*. Discussion of semi-male-sterile types will be largely limited to a consideration of a heterozygous condition with regard to the factors *X* and *Z*. Semi-male-sterile beets derived from seed borne by male-sterile S *x x z z* females could not be homozygous for *X* or *Z*.

DESCRIPTION

COMPLETELY MALE-STERILE BEETS

The female-fertile but completely male-sterile beets (S *x x z z*) bear white, empty anthers (fig. 1) and may usually be identified before the flowers open by cutting into well-developed buds. Limited observations indicated that pollen mother cells and apparently normal microspores were formed, but the microspores failed to develop and by the time the flowers opened they had largely disintegrated. No detailed study was made of the disintegration of the microspore cells.

SEMI-MALE-STERILE BEETS OF TYPE 1

Beets of semi-male-sterile type 1 (S *X x z z* or S *x x Z z*) produce yellow anthers, which usually do not dehisce; or, if they dehisce, most of the pollen grains adhere to the anther. In most instances the pollen grains produced are small (fig. 2, *B*) and nonviable, but small environmental variations cause relatively large differences. Under unfavorable environmental conditions these beets may bear pale-yellow and almost completely empty anthers. Under certain environmental conditions, not yet well defined, these semi-male-sterile beets may bear white, empty anthers and thus be indistinguishable from completely male-sterile beets. Under exceptionally favorable environmental conditions they may produce anthers fairly well filled with pollen grains, some of which may be viable.

SEMI-MALE-STERILE BEETS OF TYPE 2

Offspring from male-sterile (S *x x z z*) females that produced too much pollen to be classed as semi-male-sterile type 1 were arbitrarily designated semi-male-sterile type 2 and considered to be of constitution S *X x Z z*. In some instances it was suspected that more than two genetic factors might be involved, but proof for such an assumption must await additional results. The appearance of beets of semi-

male-sterile type 2 was markedly affected by small environmental variations, and sometimes there was much variation in the amount of pollen abortion on a single beet. When these beets, believed to be carrying S cytoplasm, could not be distinguished from normal hermaphrodites, a knowledge of their female parentage proved to be a safe criterion by which to determine their constitution and predict their breeding behavior.

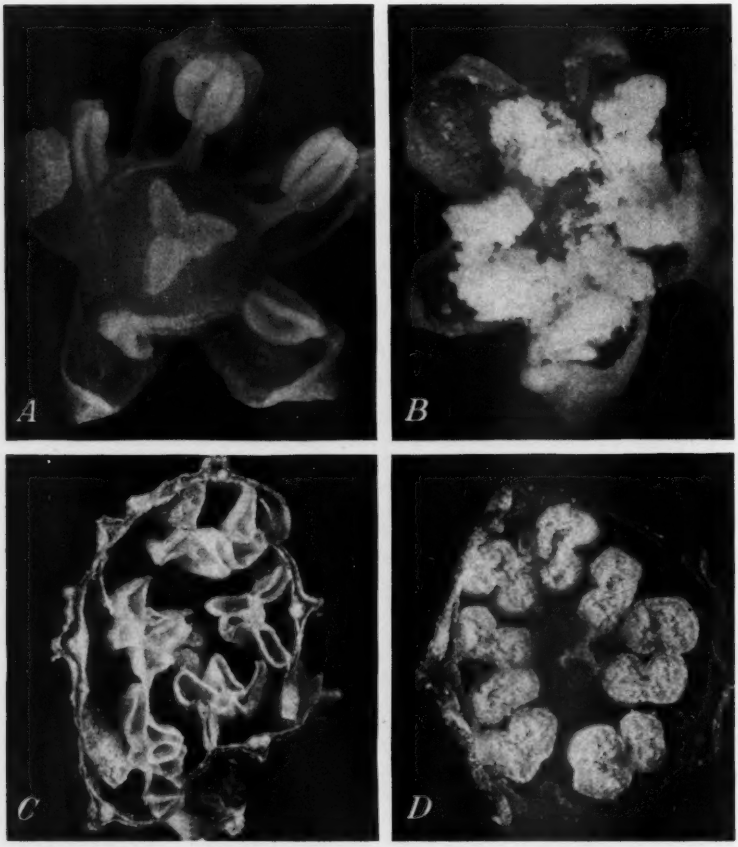

FIGURE 1.—Sugar-beet flowers. *A*, Male-sterile; × 12. *B*, Normal, immediately after dehiscing; × 12. *C*, Cross section of male-sterile flower in bud stage; × 21. *D*, Cross section of normal flower in bud stage; × 21.

EXPERIMENTAL RESULTS

RECIPROCAL CROSSES BETWEEN SEMI-MALE-STERILE AND SELECTED HERMAPHRODITE BEETS

If a character is to be explained by Mendelian, or genic, inheritance, the male and female parents may both influence the characteristics of the offspring, and populations obtained from reciprocal crosses should

be identical. If a character is to be explained by cytoplasmic, or maternal, inheritance, the male parent may have no influence on the characteristics of the offspring, and populations obtained from reciprocal crosses should not be identical. If cytoplasmic and genic

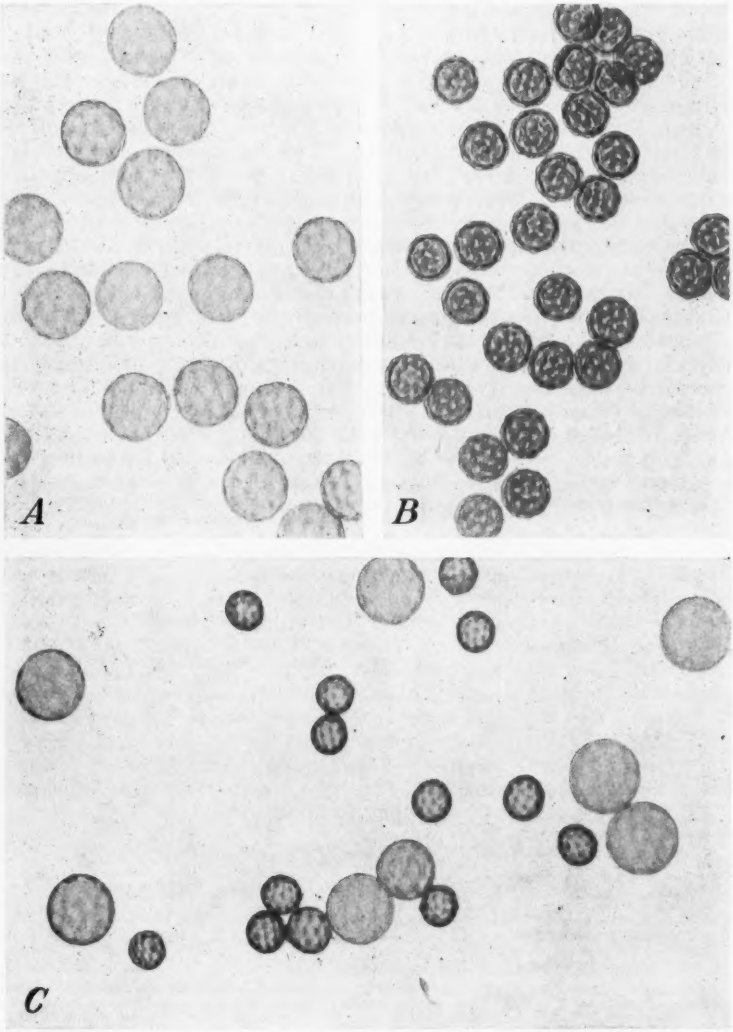

FIGURE 2.—Sugar-beet pollen: *A*, Normal; *B*, pollen from semi-male-sterile beet of type 1, with all pollen grains small and empty; *C*, pollen from semi-male-sterile beet of type 2, showing both normal and small, empty pollen grains. × 460.

inheritance are both involved in the expression of a character, the results are more complicated; but the female parent should exert more

influence than the male parent on the characteristics of the offspring, and populations obtained from reciprocal crosses should not be identical.

Reciprocal crosses could not be made with completely male-sterile beets, which produced no pollen; but many of the semi-male-sterile beets produced viable pollen, and eight reciprocal crosses were made with them. These crosses were made between six different semi-male-sterile beets and four different self-sterile hermaphrodites. The six semi-male-sterile beets differed in degree of semi-male-sterility, but a clearer discussion of these differences can be given when the effects of Mendelian factors are considered. The four hermaphrodites were selected clones with known breeding behavior. When used as pollen parents in crosses with completely male-sterile beets, these clones produced offspring nearly all of which were completely male-sterile.

The results obtained from the reciprocal crosses (table 1) were strikingly different. All the offspring grown from seed borne by the hermaphrodites, like their maternal parents, were apparently normal. The populations grown from seed borne by the semi-male-sterile beets segregated for complete male-sterility and semi-male-sterility, and in five of the populations a smaller percentage of the individuals were classified as apparently normal. This extreme difference between reciprocal crosses constitutes strong evidence for cytoplasmic inheritance. The complexity arising from the segregation in populations obtained from seed borne by the semi-male-sterile beets may be explained by assuming additional modifying effects produced by Mendelian factors.

TABLE 1.—*Reciprocal crosses between semi-male-sterile and selected hermaphrodite beets*

Parents [1]	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
	Percent	*Percent*	*Percent*	*Number*
A (semi-male-sterile) × clone 181	88	12	0	52
Reciprocal	0	0	100	111
B (semi-male-sterile) × clone 97	33	63	4	137
Reciprocal	0	0	100	43
C (semi-male-sterile) × clone 97	24	56	20	89
Reciprocal	0	0	100	15
C (semi-male-sterile) × clone 178	49	32	19	47
Reciprocal	0	0	100	22
C (semi-male-sterile) × clone 181	25	53	22	60
Reciprocal	0	0	100	84
D (semi-male-sterile) × clone 179	68	32	0	71
Reciprocal	0	0	100	74
E (semi-male-sterile) × clone 181	26	74	0	61
Reciprocal	0	0	100	119
F (semi-male-sterile) × clone 179	46	47	7	81
Reciprocal	0	0	100	41

[1] In presenting proof for cytoplasmic inheritance it is not necessary to consider the effects of two Mendelian factors assumed to be *X* and *Z*. However, the semi-male-sterile beet A was considered of constitution S *X z z z* (or S *z z Z z*) while the semi-male-sterile beets B to F were considered of constitution S *X z Z z*. The four hermaphrodites (clones 97, 178, 179, and 181) carrying normal cytoplasm were considered of constitution N *z z z z*.

HYBRIDS BETWEEN MALE-STERILE AND HERMAPHRODITE BEETS

In preliminary tests, 91 different hermaphrodites selected at random were crossed to male-sterile (S *x x z z*) females. F_1 populations from 27 of the 91 hermaphrodites were completely male-sterile, indicating that

these 27 hermaphrodites might be of constitution N *x x z z*. Definite segregation was observed in populations derived from hybridization of the male-sterile females with the remaining 64 hermaphrodites. Semi-male-sterile offspring were conspicuous in all of these populations, indicating that the male parents carried one or more Mendelian factors that influenced the effect of the S cytoplasm. The results indicated that the constitution of 31 of the parental hermaphrodites may have been S *X x z z* (or S *x x Z z*), that of 25 may have been S *X x Z z*, that of 4 may have been S *X X z z* (or S *x x Z Z*), and that of 4 may have been S *X X Z z* (or S *X x Z Z*).

Four beets, clones 97, 178, 179, and 181, which from their breeding behavior appeared to be of constitution N *x x z z*, were selected for intensive study and were maintained by vegetative propagation for a period of years. Eighteen crosses were made to male-sterile beets (S *x x z z*) with clone 97; of 841 offspring observed, all were completely male-sterile (see table 3). Fourteen crosses were made to male-sterile beets with clone 178; of 525 offspring observed, all were completely male-sterile except 4 that were semi-male-sterile. Six crosses were made to male-sterile beets with clone 179; of 442 offspring observed, all were completely male-sterile except 3 that were semi-male-sterile. The exceptional semi-male-sterile beets observed among the offspring from clones 178 and 179 were so rare that they might be accounted for by contamination. Exceptional offspring from clone 181, however, appeared to be too numerous to be accounted for by contamination; so the complete results are presented (table 2).

Environmental conditions apparently had a marked influence on the expression of the semi-male-sterile condition, because in population 0503 (table 2) 18 percent were classified as semi-male-sterile in 1940, but under slightly different environmental conditions in 1941 all offspring appeared to be completely male-sterile. Some of the segregation for semi-male-sterility might be accounted for if some of the male-sterile females crossed with clone 181 were of constitution S *X x z z* (or S *x x Z z*) instead of S *x x z z* as expected. However, this assumption could not fully explain the occurrence of semi-male-sterile beets and apparently normal beets in populations 8543 and 8550 (table 2) because, in crosses with the same females, clone 97 produced offspring all of which were completely male-sterile. This evidence may indicate that clone 181 carried a Mendelian factor not carried by clone 97 and having a smaller effect than *X* or *Z*.

SUCCESSIVE BACKCROSSES TO SELECTED HERMAPHRODITE BEETS

Successive backcrosses made by applying pollen of clone 97 to male-sterile (S *x x z z*) offspring continued to produce completely male-sterile populations (table 3). These decisive results were to be expected from the theoretical considerations, assuming clone 97 to be of constitution N *x x z z*.

It is of incidental interest that in the successive backcrosses the male-sterile offspring continued to be fully compatible with pollen from clone 97. This compatibility was also observed in 10 additional fourth-generation backcrosses made in 1943 and not shown in table 3. Insofar as they were tested, none of the male-sterile offspring showed any tendency to incompatibility with pollen from the parental clone 97. Clone 97, however, was strongly self-sterile. If a single series of allelomorphs determined self- and cross-sterility, some of the offspring

TABLE 2.—*Hybrids between male-sterile beets and clone 181*

Population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
	Percent	*Percent*	*Percent*	*Number*
8540	66	17	17	6
8543	76	14	10	21
8550	67	20	13	15
8572	100	0	0	20
8574	100	0	0	18
8622	100	0	0	15
9290	92	8	0	38
9471	98	2	0	52
0102	98	2	0	60
0103	94	6	0	54
0104	94	6	0	48
0105	98	2	0	64
0483	97	3	0	39
0485	100	0	0	52
0486	100	0	0	59
0503 [1]	82	18	0	34
0503 [2]	100	0	0	51
0511	100	0	0	35
0516	99	1	0	80
0519	100	0	0	34
0521	98	2	0	53
0533	95	3	2	57
0547A	100	0	0	45
0559	88	12	0	40
0574	92	8	0	62
0576	98	2	0	58
0579	91	9	0	54
0587	100	0	0	16
0589	97	3	0	60
0591	92	8	0	24
0593	87	13	0	54
0600	100	0	0	30
0603	100	0	0	33
0604	100	0	0	42
0607	98	2	0	42
1–559	100	0	0	105
Total observed	95.9	3.7	.4	1,570
Total expected	100	0	0	1,570

[1] Classified in 1940.
[2] Classified in 1941.

should have been incompatible with pollen from clone 97 in the second, third, and fourth backcross generations. That this incompatibility

TABLE 3.—*Hybrids between male-sterile beets and clone 97*

Generation	Populations	Offspring		
		Male-sterile	Semi-male-sterile	Apparently normal
	Number	*Number*	*Number*	*Number*
F_1	8	405	0	0
First backcross	3	158	0	0
Second backcross	4	177	0	0
Third backcross	3	101	0	0
Total	18	841	0	0

was not observed confirms previous evidence (*11*) that more than one allelomorphic series of factors is necessary to explain cross-sterility in beets.

HYBRIDS BETWEEN SEMI-MALE-STERILE AND SELECTED HERMAPHRODITE BEETS

From populations derived by crossing semi-male-sterile beets of type 1 (S *X x z z* or S *x x Z z*) with clone 97 (N *x x z z*), an excess of male-sterile offspring was obtained as compared with semi-male-sterile offspring when a 1:1 ratio was expected (table 4). Heritable factors may have caused the lack of agreement between expected and observed results, but environmental influences may be given first consideration. Under slightly different environmental conditions and with provision for closer examination, some of the apparently complete male-sterility may have been more accurately classified as semi-male-sterility. In 1 population of 71 beets (population 1–647, table 4), grown in the greenhouse to permit close periodical observations, a good agreement with the expected 1:1 ratio was observed, 49 percent being male-sterile and 51 percent semi-male-sterile.

Apparently normal offspring were observed in populations 2628 and 2631 (table 4), which would indicate female parentage of genic constitution *X x Z z*, rather than *X x z z* or *x x Z z* as expected from their appearance. The two semi-male-sterile female parents in question were from inbred lines with reduced vigor, which may account for their type 1 (S *X x z z* or S *x x Z z*) appearance but type 2 (S *X x Z z*) breeding behavior (see table 5).

TABLE 4.—*Hybrids between semi-male-sterile beets and clone 97*

Hybrid and population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
S *X z z* (or S *x Z z*) × N *x z z*:	*Percent*	*Percent*	*Percent*	*Number*
4325	92	8	0	75
5502	87	13	0	158
1–501	95	5	0	199
0497	85	15	0	47
1–629	76	24	0	68
1–631	90	10	0	68
1–647	49	51	0	71
1–668	68	32	0	76
1–669	70	30	0	77
1–682	59	41	0	164
1–696	86	14	0	70
2619	86	14	0	35
2628	53	33	14	36
2631	41	56	3	59
2635	94	6	0	33
Total	76.8	22.7	0.6	1,236
Expected 1:1 ratio	50	50	0	1,236
S *X z Z z* × N *x z z*:				
1–648	60	38	2	55
1–649	33	63	4	137
1–685	43	41	16	63
1–686	24	56	20	89
1–693	58	37	5	79
2580	37	63	0	19
Total	40	60		442
Expected 1:3 ratio	25	75		442

From populations derived by crossing beets of semi-male-sterile type 2 (S *X x Z z*) with clone 97 (N *x x z z*), the observed results are in relatively close agreement with expected results (table 4), considering the nature of the environmental variability. The excessive number of individuals classified as completely male-sterile might be explained by environmental influence or by failure to make sufficiently

close examinations. It was sometimes difficult to distinguish semi-male-sterile beets from apparently normal hermaphrodites, so no close distinction should be made between these two classes. Both of these classes were expected, and furthermore the difficulty in distinguishing between them was expected.

Beets of semi-male-sterile types 1 and 2 were crossed with pollen from the additional hermaphrodite (N *x x z z*) clones 178, 179, and 181, and the results were similar to those obtained when clone 97 was used as the pollen parent. Observations of offspring from one of the hybrids with a semi-male-sterile (type 1) female and from five hybrids between semi-male-sterile (type 2) females and clones 178, 179, or 181 are shown in table 1 in connection with the presentation of results with reciprocal crosses.

HYBRIDS BETWEEN MALE-STERILE BEETS AND CLONE 90

The preliminary results indicated that the hermaphrodite designated clone 90 might be homozygous, with a constitution represented by S *X X z z* or S *x x Z Z*. Observations from eight F_1 populations were eventually obtained after eight different crosses between male-sterile females and clone 90 had been made.

All the F_1 offspring were expected to be semi-male-sterile because they were assumed to be of constitution S *X x z z* or S *x x Z z*. These were essentially the results obtained in seven of the eight populations (table 5). Under favorable environmental conditions the beets in these seven populations were very nearly uniform in degree of semi-male-sterility. The anthers were yellow, but very little pollen dehisced and none appeared to be viable. In population 2522 (table 5), 43 beets were classified and discarded by May 6, 1943. These were very nearly uniform in degree of semi-male-sterility. By May 24, 1943, during a period with higher temperatures, 15 more beets in this population came into flower. The degree of male-sterility of all these 15 beets was definitely more pronounced, 4 of them being completely male-sterile, with white anthers.

TABLE 5.—*Hybrids between male-sterile beets and clone 90*

Hybrid and population No.	Offspring			
	Male-sterile	Semi-male-sterile [1]	Bearing some pollen	Total
S *x x z z* × N *X X z z* (or N *x x Z Z*):	*Percent*	*Percent*	*Percent*	*Number*
4344	11	89	0	9
4634	8	92	0	73
8568	7	93	0	15
1–566	24	68	8	74
1–573	8	92	0	63
2466	3	97	0	30
2522	7	93	0	58
2562	2	98	0	54

[1] Nearly all the semi-male-sterile offspring were very nearly uniform in appearance. The anthers were yellow and partially filled, but they seldom dehisced and apparently bore no viable pollen.

Results with population 1–566 (table 5) failed to confirm the results obtained with the other seven populations. Observations of 74 beets grown from this population showed that 24 percent were completely male-sterile, 68 percent were semi-male-sterile, and 8

percent produced more or less normal pollen. Environmental conditions were favorable for flowering and pollen development, so the cause of the relatively wide segregation in population 1–566 was believed to be heritable rather than environmental. To explain these exceptional results, it would be necessary to assume that the male-sterile (S *x x z z*) female parent carried two additional Mendelian factors, one increasing and the other decreasing the degree of male-sterility.

HYBRIDS BETWEEN MALE-STERILE AND SEMI-MALE-STERILE BEETS

It has been assumed that semi-male-sterile beets carry the same cytoplasm as completely male-sterile beets. The difference between male-sterile and semi-male-sterile beets is attributed to one or more Mendelian factors carried by the semi-male-sterile plants. If this assumption is correct, the Mendelian factors in question should be carried by any viable pollen produced by the semi-male-sterile plants. Consequently, a male-sterile (S *x x z z*) female crossed with any type of semi-male-sterile beet should produce some semi-male-sterile offspring. In no case should all the offspring be completely male-sterile, indicating male parentage of genic constitution *x x z z*.

Two crosses of the type S *x x z z* × S *X x z z* were made with the poor pollen from semi-male-sterile beets of type 1. Six crosses were made between male-sterile (S *x x z z*) beets and semi-male-sterile beets considered to be of type 2 and of constitution S *X x Z z* (table 6).

Since each of the eight populations (table 6) segregated for complete male-sterility and semi-male-sterility, the observed results are in essential agreement with expected results. The recovery of some apparently normal beets from the crosses with semi-male-sterile beets of type 2, used as pollen parents, was to be expected. If these apparently normal beets had been subjected to further study over a period of time, it is possible that they would have shown a tendency to semi-male-sterility.

TABLE 6.—*Hybrids between male-sterile and semi-male-sterile beets*

Hybrid and population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
S *x x z z* × S *X x z z* (or S *x x Z z*):	*Percent*	*Percent*	*Percent*	*Number*
335	63	37	0	19
1-549	57	43	0	14
Total	61	39	0	33
Expected 1:1 ratio	50	50	0	33
S *x x z z* × S *X x Z z*:				
1-538	62	36	2	117
1-583	22	54	24	72
1-607	24	72	4	100
1-610	53	41	6	32
1-618	42	34	24	157
2470	58	38	4	26
Total	42	58		504
Expected 1:3 ratio	25	75		504

SEMI-MALE-STERILE BEETS SELFED

Male-sterile and semi-male-sterile beets apparently carrying S cytoplasm were crossed with hermaphrodites carrying a factor S^f for

self-fertility. From results of breeding behavior, it was eventually revealed that six of the self-fertile hermaphrodites used as pollen parents could be considered of constitution N *X x Z z* with regard to inheritance of male-sterility. These six self-fertile (N *X x Z z*) hermaphrodites were used as pollen parents in crosses with both male-sterile and semi-male-sterile females. Wide segregations were observed in F_1 populations. F_2 populations were obtained by selfing selected self-fertile F_1 beets that produced sufficient viable pollen. The results are presented in table 7, the F_2 populations being grouped according to the classification of their respective female grandparents.

TABLE 7.—*Offspring of selfed beets carrying* S *cytoplasm, with pollen grandparents considered of constitution* N *X x Z z and female grandparents as indicated*

Grandparents		Self-fertilized population No.	Offspring			
Female	Male (N *X x Z z*)		Male-sterile	Semi-male-sterile [1]	Bearing some pollen [2]	Total
Male-sterile (S *x x z z*):			*Percent*	*Percent*	*Percent*	*Number*
Group	9508-1	1-752	44	26	30	27
	9508-1	1-753	42	42	15	33
	9508-1	1-754	51	31	18	93
	9508-1	1-755	39	50	11	38
9148-4	0448-48	2267	39	49	12	43
	0448-48	2268	20	70	10	110
	0448-48	2269	27	29	44	52
	0448-48	2270	20	36	44	50
	0448-48	2271	40	47	13	15
	0448-48	2272	14	48	38	21
0579-151	0448-48	2273	26	31	43	35
Semi-male-sterile (S *X x z z* or S *x x Z z*):						
8114-105	0475-13	2263	7	93	0	28
	0475-15	2264	12	88	0	83
	0475-20	2265	0	90	10	99
	0475-20	2266	20	80	0	41
046-1606	0475-20	2274	15	65	20	106
	0475-20	2275	2	60	38	105
Semi-male-sterile (S *X x Z z*):						
046-1121	0475-20	2276	0	38	62	69
	0475-20	2277	7	49	44	105
	0475-20	2278	0	79	21	123
	0475-20	2279	1	70	28	67
0107-4	9254-1	2280	0	24	76	25
0493-1	0475-13	2281	0	0	100	58

[1] Offspring classified as semi-male-sterile bore yellow anthers but apparently no viable pollen.
[2] Some offspring classified as bearing pollen showed evidence of being semi-male-sterile, but no clear distinction could be made between this condition and the normal hermaphrodite condition.

In the first group (table 7), where the grandparents were considered to be S *x x z z* and N *X x Z z*, all 11 of the observed self-fertilized populations showed wide segregation, some beets being completely male-sterile, some semi-male-sterile, and others relatively normal. In the second group, where the grandparents were considered to be S *X x z z* (or S *x x Z z*) and N *X x Z z*, all 6 of the observed self-fertilized populations showed segregation, but the average percentage of male-sterile beets was less than in the first group. In the third group, where the grandparents were considered to be S *X x Z z* and N *X x Z z*, the 6 observed self-fertilized populations showed definitely less segregation, population 2281 showing no segregation. The beets in population 2281 were uniform with regard to pollen production. The amount of pollen was perhaps less than normal, but microscopic examinations failed to show any consistent difference between the pollen from these beets and that pollen produced by normal hermaphrodite beets.

Crosses have been made between male-sterile (S *x x z z*) females and self-fertile beets considered to be of possible constitution S *X X Z Z*, like the self-fertile beets in population 2281. From these crosses it is expected that more information may be obtained regarding the Mendelian factors that influence the degree of male-sterility in the presence of S cytoplasm.

OPEN POLLINATIONS

As a rule the most critical genetic information is obtained from controlled crosses where the male parent, as well as the female parent, is known. With cytoplasmic inheritance, however, it is possible to obtain critical information regarding type of cytoplasm by open pollination to a heterogeneous source of pollen.

Seven apparently normal beets grown from seed borne by male-sterile (S *x x z z*) females were investigated to determine whether they carried S cytoplasm as expected. These seven beets were grown in three different beet-seed isolations. They showed no sign of semi-male-sterility during repeated examinations. Each beet was tagged for identification during flowering and was allowed to open-pollinate with the mass of unknown pollen to which it was exposed. The results from the populations grown from the open-pollinated seed borne by the seven apparently normal beets are shown in table 8. All seven of the populations produced male-sterile and semi-male-sterile offspring as well as variable percentages of apparently normal offspring. This is ample proof that the seven apparently normal female beets carried S cytoplasm. If they had carried N cytoplasm, all the offspring should have been normal.

TABLE 8.—*Offspring derived by open pollination from apparently normal beets carrying S cytoplasm*

Population No.	Offspring			
	Male-sterile	Semi-male-sterile	Apparently normal	Total
	Percent	*Percent*	*Percent*	*Number*
0111	39	37	24	38
1–117	27	24	49	66
1–118	37	53	10	90
1–119	18	58	24	91
1–120	6	60	34	67
1–135	14	64	22	92
1–136	54	39	7	56
Total	26	50	24	500

CYTOPLASMIC INSTABILITY

Substances carried by the cytoplasm and responsible for cytoplasmic inheritance appear to be relatively stable in some instances and relatively unstable in others. When a chlorophyll deficiency is brought about by cytoplasmic inheritance it is not uncommon in several species of plants (*15*) to see sectorial arrangements, some branches being green, others white, and others partly green and partly white. An unstable chlorophyll condition of this type, believed due to cytoplasmic inheritance, has been described in beets (*8*, *13*). After extensive investigations with cytoplasmic inheritance

in *Epilobium*, Michaelis (*7*) stated that the substances carried by the cytoplasm that are responsible for cytoplasmic inheritance are much less stable than the genes carried by the chromosomes. A somewhat different opinion is expressed by Lewis (*6, p. 61*), as follows:

> Cytoplasmic mutations appear to occur more rarely than gene mutations; however, it is probable that changes in the cytoplasm arise more frequently than the data reveal, but are not observed because the effects are so slight.

The writer's observations would indicate that the cytoplasmically inherited male-sterility in beets is a relatively stable character. However, some unexplained results might be accounted for by cytoplasmic instability, and in 2 instances the instability was definite and of striking degree. In the course of routine classification work, 2 beets from rather widely separated parentage were observed to be highly variable. Most of the inflorescence of each of these beets showed complete male-sterility, but some sectors showed semi-male-sterility, occasional anthers producing viable pollen. One of these variable beets was self-fertile, and observations were continued by growing its offspring with population 2612, obtained from self-pollinated seed, and with population 2613, obtained from open-pollinated seed produced by the same plant. Twenty-six offspring were grown from population 2612 and 38 from population 2613. All these offspring were observed to be completely male-sterile, except 9 individuals (2 from population 2612 and 7 from population 2613) which exhibited an unstable condition similar to that of the maternal parent. On these 9 offspring, viable pollen was produced by some branches of inflorescences, although most of the branches bore anthers that were white and completely male-sterile. Variability upon single branches was also observed, and in a few instances empty, white anthers and yellow anthers filled with pollen were borne within the same flower.

The maternal beet of populations 2612 and 2613 might be considered of genic constitution *x x z z* because of the high proportion of complete male-sterility observed in the offspring. This parental beet and those of the offspring also showing the unstable condition evidently carried S cytoplasm, but the evidence indicates that it was not the usual kind of S cytoplasm. Apparently some change in its stability had taken place. The sectors bearing viable pollen might be considered of constitution N *x x z z*, whereas most of the male-sterile portion of the beet might be considered of constitution S *x x z z*.

MALE-STERILITY NOT CYTOPLASMICALLY INHERITED

Male-sterility apparently similar to that already described but with a different mode of inheritance was encountered in two different varieties of beets. One variety was the 12c strain (*11*), a noncommercial curly-top-resistant variety, and the other was the Munerati annual variety (*9*). Both of these varieties had apparently been derived by relatively close breeding, and occasional male-sterile beets were observed in both.

Two male-sterile beets, 841–9 and 841–10, from the Munerati annual variety were crossed with the well-proved N *x x z z* hermaphrodite clones 97 and 179. A male-sterile beet designated 3252–276, from the 12c strain, was crossed with pollen from clone 97. All the offspring (table 9) appeared to be normal hermaphrodites with a heavy production of pollen and without any indication of male-

sterility or semi-male-sterility. If the male-sterile female parents had carried S cytoplasm, all the offspring should have been male-sterile. The evidence, therefore, indicates that the male-sterility in the 12c strain and in the Munerati annual variety must have been produced by a different mode of inheritance. This inheritance may have been entirely genic, without cytoplasmic influence.

TABLE 9.—*Hybrids between exceptional male-sterile and selected hermaphrodite beets*

Population No.	Male-sterile female	N *x z z z* normal male	Offspring		
			Male-sterile	Semi-male-sterile	Apparently normal
			Number	*Number*	*Number*
2855	841-9	Clone 97	0	0	21
2856	841-9	Clone 179	0	0	19
2859	841-10	do	0	0	21
4789	3252-276	Clone 97	0	0	9

In inbred lines of beets, the writer has observed additional instances of male-sterility where the character is probably independent of cytoplasmic inheritance. Male-sterility has been common in inbred lines, even where there was good evidence that the lines carried N rather than S cytoplasm.

UTILIZATION OF MALE-STERILITY IN HYBRIDIZATION WORK

The cytoplasmically inherited male-sterility in beets offers unique possibilities for obtaining a wholesale emasculation of flowers to facilitate hybridization work. Controlled crosses make it possible to produce male-sterile offspring in whatever quantity is desired, either for small-scale genetic studies or for field crosses for the production of hybrid seed. It has been shown that, with few exceptions, crosses of the type S *x z z z* × N *x z z z* produce offspring all of which are completely male-sterile. Therefore, obtaining a quantity of seed that can be relied upon to produce completely male-sterile offspring is dependent upon selecting a sufficient number of hermaphrodites that may be considered of constitution N *x z z z*.

Hermaphrodites that may be considered of constitution N *x z z z* appear to be fairly common in curly-top-resistant varieties of sugar beets, but they can be identified only by breeding behavior. The N *x z z z* hermaphrodites were propagated in three ways: (1) By clonal or asexual propagation (*10*), (2) by introducing a factor for self-fertility (*11*) and inbreeding, and (3) by reproducing offspring obtained by intercrossing self-sterile N *x z z z* clones with proved breeding behavior. Vegetative propagation alone has only limited applications, although such propagation helps materially in holding clones until their breeding behavior is known. The inbreeding method has great potentialities, but inbred lines of sugar beets with satisfactory resistance to curly top are only now beginning to be made available. Therefore, the third method, namely, working with intercrosses between self-sterile beets that may be considered of constitution N *x z z z* has been the principal method of propagation used by the writer.

The self-sterile clone 97, whose breeding behavior indicated the N *x z z z* constitution, was hybridized with other self-sterile (N *x z z z*) clones with similar breeding behavior. The self-sterile F_1 populations

were reproduced by maturing seed by open pollination in isolated field plantings. Hybrids between selected male-sterile (S *x x z z*) beets and the N *x x z z* populations made it possible to obtain an abundance of seed that could be depended upon to produce completely male-sterile offspring with but very few exceptions. The exceptional offspring, although not completely male-sterile, generally produced no viable pollen. Finally a field planting was made in which 16-row strips of male-sterile beets were alternated with 4-row strips of a normal hermaphrodite variety (fig. 3). With this planting arrangement, pollination by insects or by wind appeared to be adequate because the acre yield of seed from the male-sterile strips was about 3,000 pounds, approximating or slightly exceeding the yield from the strips of the normal variety.

FIGURE 3.—Sugar-beet seed field in which 16-row strips of male-sterile sugar beets were alternated with 4-row strips of a normal variety. The color contrast was due to the male-sterile beets being a darker green than the normal pollen-bearing beets.

Excellent male-sterile breeding stock was produced in this manner, but no attempt is being made to hasten commercial applications for the production of hybrid seed until the underlying research work, including work with inbred lines, has made further progress.

DISCUSSION

The findings with respect to combined effects of cytoplasmic and genic inheritance make it apparent that caution should be used in attributing all the influence to either of these two modes of inheritance. This need for caution may be illustrated by the male-sterility in the curly-top-resistant sugar beets where male-sterile beets may be considered of constitution S *x x z z*, S representing a particular type of cytoplasm and *x x z z* representing the complementary genic constitution. Observations restricted to offspring from hybrids between male-sterile S *x x z z* females and N *x x z z* hermaphrodites would lead to the assumption of cytoplasmic inheritance without genic influence. Observations restricted to offspring derived by selfing beets of constitution S *X x Z z*, with the degree of male-sterility determined by the recombination of the Mendelian factors *X* and *Z*, would lead to the assumption of genic inheritance without cytoplasmic influence. A comprehensive understanding of the combined effect

of the two modes of inheritance can be gained only by devising tests for both the cytoplasmic inheritance and the genic inheritance.

It is of interest that in corn (*Zea mays*), although Mendelian factors for male-sterility have been frequently reported (*5*), cytoplasmically inherited male-sterility has not been reported except from limited sources in South America. Gini (*5*) investigated different sources and modes of inheritance of male-sterility in *Z. mays* found in Argentina and traced all the cytoplasmically inherited male-sterility to a single region, the Province of Entre Ríos. Rhoades (*14*) reported another case of cytoplasmically inherited male-sterility in the offspring of *Z. mays* collected at Arequipa, Peru.

SUMMARY AND CONCLUSIONS

Male-sterility produced by combined cytoplasmic and genic inheritance was found in cross-pollinated varieties of sugar beets bred for resistance to curly top. Complete male-sterility was characterized by white, empty anthers. The inheritance of semi-male-sterility was found to be related to that of complete male-sterility. Assuming two types of cytoplasm, S for male-sterility and N for normal, and two Mendelian factors *X* and *Z*, the majority of the evidence, including striking differences from reciprocal crosses, indicates the following constitution of male-sterile and heterozygous semi-male-sterile beets:

S *x x z z*=completely male-sterile.
S *X x z z* (or S *x x Z z*)=semi-male-sterile, usually without viable pollen.
S*X x Z z*=semi-male-sterile, usually with some viable pollen and sometimes indistinguishable from normal hermaphrodite.

Occasional results were not fully explained by the two-factor hypothesis, indicating that more than two Mendelian factors may influence the degree of semi-male-sterility. Instances of homozygosity for the Mendelian factors were demonstrated, but offspring from male-sterile (S *x x z z*) females were never found to be homozygous for *X* or *Z*.

The male-sterile character in the curly-top-resistant varieties of beets appeared to be relatively stable, but in two instances cytoplasmic instability was encountered, single male-sterile beets producing sectors of inflorescence bearing more or less normal anthers with viable pollen. Seed from beets in this unstable condition produced offspring some of which continued to show striking degrees of instability.

The male-sterile sugar beets produced by combined cytoplasmic and genic inheritance were found to be especially convenient for use as female parents in hybridization work, because the nature of the inheritance makes possible the production of completely male-sterile populations. With but very few exceptions, male-sterile (S *x x z z*) females crossed with selected N *x x z z* hermaphrodites produced completely male-sterile offspring.

Evidence indicates that some types of male-sterility in beets are produced by genic rather than cytoplasmic inheritance. Male-sterility found in the Munerati annual beet and in the curly-top-resistant strain 12c did not appear to be inherited cytoplasmically.

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RESISTANCE IN POTATO VARIETIES TO YELLOW DWARF[1]

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INTRODUCTION

The yellow dwarf disease of potato (*Solanum tuberosum* L.)was of little concern in Wisconsin before 1930, but during the years 1930, 1931, and 1932 it increased to such an extent in the central part of the State, especially in the counties of Portage, Waupaca, and Waushara, that it became the most important virus disease and in many cases the limiting factor in potato production. The disease was also very severe in Washington County in southeastern Wisconsin in 1935. In 1937 it was again of major importance in the State, not only in the central part but throughout Fond du Lac, Outagamie, Racine, and Washington Counties. Yellow dwarf is also present, although to a lesser extent, in the northwest section in Barron, Polk, and Washburn Counties and in the northeastern section in Door, Marinette, and Oconto Counties. The endemic occurrence and sporadic appearance of the disease renders it a constant hazard to the late potato crop. The loss sustained by the growers consists not only in decreased yields of crops grown for table stock but also in the added cost of replacing the virus-infected seed stock.

When infected seed stocks were recognized as the most important source of virus infection, the yearly planting of seed from yellow-dwarf-free areas was recommended as a control measure for central Wisconsin. The results were very encouraging when this practice was followed. However, the cost of seed is an important factor with the majority of small growers and the replanting of locally grown seed stock continued to be a common practice. This replanting of infected seed furnished a reservoir for yellow dwarf infection of other introduced virus-free potato stocks. Moreover, in many instances during a season of extensive spread of the virus, plants from disease-free seed accumulated enough of the virus to make the crop unprofitable.

Since the heavy losses in the zones where yellow dwarf commonly builds up rapidly could be materially reduced by the increased use of a late potato variety tolerant to the virus and adapted to the area, the existence of resistance in standard potato varieties has been studied since 1937. The first reference to the susceptibility of potato varieties to the yellow dwarf virus was made by Barrus and Chupp (*2*) [2] in New York in 1922. They observed the disease in 18 varieties of potatoes and reported that none of them showed any degree of resistance. Fernow and Black (*4*) in 1932 reported yellow dwarf more abundant in the Green Mountain than in the Rural variety. Muncie (*8*) in 1935 asserted that all commercial varieties of potatoes grown in

[1] Received for publication February 3, 1944.

[2] Italic numbers in parentheses refer to Literature Cited, p. 451.

Michigan were susceptible but made no mention of differences in disease incidence. A year later, Wheeler (*12*) reported that all the commercial potato varieties grown in Michigan showed a high percentage of the disease but that several potato-seedling varieties under test showed resistance to infection under natural field conditions and under artificial conditions by plug-graft inoculations. Black (*3*) in 1937 reported from New York that no immune potato plant had yet been discovered. In 1938, Taylor (*10*) found in a variety test planting in New York that the Columbia Russet variety was the most severely infected and Russet Burbank the least infected of 7 varieties when exposed to natural field infection. In a preliminary report on field resistance Walker and Larson (*11*) reported in 1939 that of 19 varieties or strains of potato that had been exposed to natural field infection in central Wisconsin, the Russet Burbank showed the lowest incidence of the disease. Hansing (*5*, *6*), reporting in 1942 and 1943, stated that in several replicated field experiments conducted in New York the varieties Arran Banner, Chippewa, Golden, Houma, Jubel, Katahdin, Sebago, and Warba had less than 2 percent of yellow-dwarf-infected plants while Russet Burbank had 2 to 11, Rural 2.9 to 21.1, Russet Rural 4.2 to 29.1, Green Mountain 6 to 53, Early Bovee 18 to 28, and Red McClure 51.6 percent, respectively. He also stated that in 20 United States Department of Agriculture potato seedling varieties under test, the percentage of infected plants ranged from 0 to 72. Recently, Rieman and McFarlane (*9*) reported that yellow dwarf was more severe in Russet Rural than in Sebago during 1941.

EXPERIMENTAL RESULTS

FIELD EXPERIMENTS

1937–38 Trials

In 1937, 19 certified varieties or strains of potatoes were grown as an adaptation planting in a plot of 4 randomized replicated blocks situated 2 miles north of Almond, Portage County, Wis. This planting was one of the 7 potato variety adaptation plantings set up in the State that year by J. W. Brann, of the Departments of Horticulture and Plant Pathology of the University of Wisconsin. Pronounced primary yellow dwarf symptoms were observed about the middle of August on a large number of plants in this planting. The current-season symptoms were not confined to any one variety or strain but were scattered throughout the planting. A small percentage of secondarily infected plants were found in one of the varieties early in the season. This small amount of virus, however, was not entirely responsible for the pronounced spread of disease since the adaptation planting was surrounded on 3 sides by an 8-acre commercial field of Russet Rural potatoes in which 3 percent of the plants showed secondary yellow dwarf symptoms early in the season (fig. 1). It was observed during early September that the varieties in the adaptation planting exhibited marked variation in the incidence of primary yellow dwarf symptoms, although such differences could not be accurately recorded.

The spread of the yellow dwarf virus in the Almond area and in most of the central Wisconsin commercial potato section generally during the growing season of 1937 was very pronounced. A count of

yellow dwarf in the Russet Rural planting surrounding the adaptation planting in the latter part of the growing season showed primary symptoms on about 65 percent of the plants, and sweepings revealed

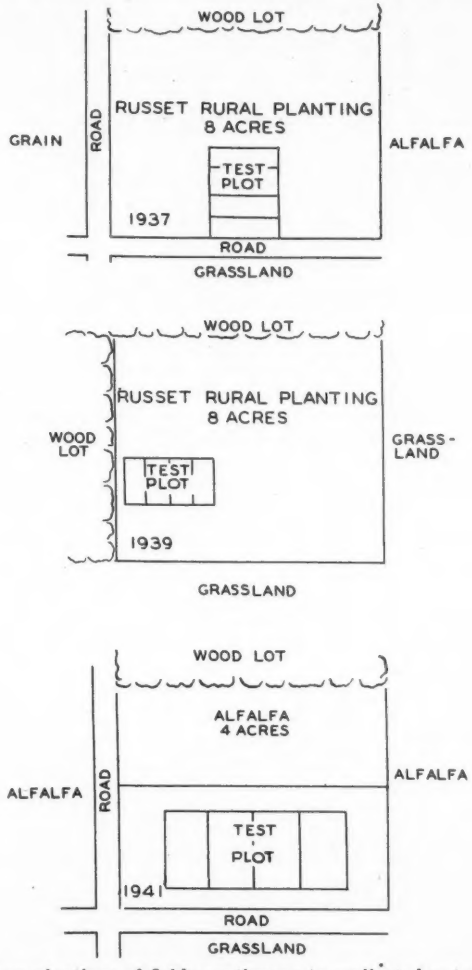

FIGURE 1.—Crop plantings of fields contiguous to replicated potato variety test blocks in a plot in central Wisconsin, near Almond, Portage County, 1937, 1939 and 1941.

a very large population of the clover leafhopper (*Aceratagallia sanguinolenta* (Prov.).

After harvest of the potatoes in the adaptation planting a random sample of U. S. No. 1 size tubers from a composite of the 4 replicates of each of the 19 varieties was placed in a common storage. A 40-tuber sample from each composite was obtained and planted as

wholetuber seed pieces at Hancock, Wis., in early May 1938. The detectable secondary yellow dwarf symptoms and nonemerging hills resulting from tuber infection were recorded and the tabulated results are presented in table 1.

It will be noted that the late-maturing Russet Burbank and the early-maturing Warba (pink-eyed) showed the lowest percentage when both nonemerging hills and secondary yellow dwarf symptoms are considered. Russet Rural and Triumph were the most severely infected of the late- and early-maturing varieties, respectively.

TABLE 1.—*A comparison of the incidence of the yellow dwarf virus as shown by secondary field symptoms during each succeeding season in certain varieties and strains of potato grown under natural field conditions in randomized replicate blocks in the high-disease zone near Almond, Wis., in 1937, 1939, and 1941*

Varieties under test	1937–38			1939–40			1941–42			1937–38, 1939–40, 1941–42		
	Nonemerging hills	Secondary yellow dwarf hills	Total nonemerging and secondary yellow dwarf hills	Nonemerging hills	Secondary yellow dwarf hills	Total nonemerging and secondary yellow dwarf hills	Nonemerging hills	Secondary yellow dwarf hills	Total nonemerging and secondary yellow dwarf hills	Average nonemerging hills	Average secondary yellow dwarf hills	Average total nonemerging and secondary yellow dwarf hills
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Russet Rural	43	33	76	38	60	98	6	45	51	29	46	[1]75xxx
White Blossom Cobbler [2]	68	5	73							68	5	73x
Triumph	53	28	81	20	76	96	4	32	36	26	45	71xxx
Toanco [3]	48	23	71							48	23	71x
Mesaba	30	40	70							30	40	70x
Green Mountain	23	47	70	4	66	70	8	47	55	12	53	65xxx
Earlaine	38	23	61							38	23	61x
Rural New Yorker	38	13	51	28	56	84	3	29	32	23	33	56xxx
Columbia Russet	13	38	51							13	38	51x
Pioneer Rural [4]	20	30	50							20	30	50x
Pontiac							0	44	44	0	44	44x
Katahdin	13	40	53	8	42	50	4	23	27	8	35	43xxx
Houma	15	38	53	0	32	32				8	35	43xxx
Chippewa	8	40	48	6	38	44	1	17	18	5	32	37xxx
Irish Cobbler	18	28	46	0	38	38	3	15	18	7	27	34xxx
One Hundred Day Cobbler	15	18	33							15	18	33x
Sequoia							5	19	24	5	19	24x
Russet Burbank	5	13	18				2	12	14	6	13	19xx
Warba [5]	5	23	28	0	10	10	3	5	8	3	13	16xxx
Sebago				2	4	6	2	4	6	2	4	6xx

[1] x=1-year test; xx=2-year test; xxx=3-year test.
[2] Natural seedling of Cobbler.
[3] Strain of Rural New Yorker.
[4] Smooth mutant of Russet Rural.
[5] 1937–38, Pink-eyed Warba; 1939–40 and 1941–42, Red Warba.

1939–40 TRIALS

The opportunity for further studies of varietal resistance in the potato to the yellow dwarf virus under natural field conditions was again afforded during the season of 1939. An early-season examination of the large commercial planting (fig. 1) of the Russet Rural variety 1 mile south of Almond, surrounding the central Wisconsin varietal adaptation planting, was found to contain about 6 percent of secondary yellow dwarf plants, and the insect vector (*A. cerata-gallia sanguinolenta*) was present in considerable numbers. This potato variety adaptation planting was one of 9 field plantings set up

in 1939 at widely distributed points in the State by G. H. Rieman, of the Departments of Genetics, Horticulture and Plant Pathology, University of Wisconsin. The 10 varieties in the adaptation planting were either of certified seed or of foundation seed stock free of the yellow dwarf virus and were planted in 4 blocks in a plot in which the arrangement of varieties was randomized. Red Warba (*7*) was substituted in this trial for the pink-eyed Warba. A late-season count in the commercial Russet Rural planting surrounding the adaptation planting showed primary yellow dwarf symptoms on about 72 percent of the plants, indicating a very general spread of the virus in this planting. In the adaptation planting, primary yellow dwarf symptoms were observed throughout the replicated blocks.

A 50-tuber sample of U. S. No. 1 size from the composites of each variety in the adaptation planting were obtained and planted in 100-hill, single-row, 2-hill-per-tuber units at Almond in late May 1940. The occurrence of secondary yellow dwarf and of nonemerging hills were recorded in detail during the season. The data are presented in table 1.

When percentages of nonemerging hills and of secondary yellow dwarf were considered, Russet Rural and Triumph again showed the highest incidence of disease. Red Warba and Sebago were least affected. It is significant to note that there was again no correlation between time of maturity of variety and resistance, since the two most resistant varieties were in one case a very early maturing one (Warba) and in the other a very late maturing one (Sebago).

1941–42 Trials

Because of the differences in disease incidence exhibited by certain potato varieties in the two earlier field plantings, it was important to know whether varieties that showed low incidence of secondary yellow dwarf after being subjected to severe infection under natural conditions, would continue to show resistance when disease-free seed of these varieties was again exposed to severe natural virus infection.

In the spring of 1941 a test planting was made in the field previously used for the 1937 adaptation planting (fig. 1). However, 4 acres of alfalfa had replaced half of the Russet Rural planting area, and across the road alfalfa had replaced the grain. Twelve yellow-dwarf-free varieties (certified or foundation stock) were planted in 4 randomized blocks of 25 hills each. To insure as uniform infection as is possible under field conditions, 4 rows of yellow-dwarf-infected Russet Rural seed tubers (rows 2 to 5, 7 to 10, 12 to 15, etc.) were planted between each variety row; thus all test varieties were immediately adjacent to plants growing from virus-infected seed, as shown in figure 2. In addition, the entire test plot was surrounded by 30 rows of infected seed tubers. The yellow-dwarf-infected seed tubers planted as inoculum were selected in the spring of 1941 from a Russet Rural seed stock grown for two successive seasons in an area of central Wisconsin where yellow dwarf was prevalent. This seed stock was found to contain about 27 percent of secondary yellow-dwarf-infected plants early in 1940 and when examined later that season showed about 60 percent primary infection.

In the Almond area during the 1941 season insect sweepings in commercial potato fields, along roadsides, and fence rows, in grass-

lands and in waste areas showed a large population of the yellow-dwarf vector. A field count of primary disease symptoms in commercial potato plantings in this area and in the Russet Rural planting surrounding the test varieties indicated a pronounced current season spread of the virus. A late-season survey of the varietal test planting showed widespread current-season infection. At harvest a 300-pound random sample of U. S. No. 1 size tubers was collected from each composite of the four replicates of each variety grown in the test planting and placed in a common storage at Almond for the 1942 field planting.

FIGURE 2.—Planting arrangement of test varieties and yellow-dwarf-infected Russet Rural potatoes at Almond, Portage County, Wisc., 1941.

A random 200-tuber sample of each composite of the 12 varieties was selected in May 1942, and planted at Almond as whole-tuber seed pieces in 100-hill, 2-row plots, in early June. The nonemerging hills and detectable secondary yellow dwarf symptoms were recorded during the season and the data as to the rate of degeneration after infection in the 12 standard varieties under test are recorded in table 1.

The marked variation in incidence of disease between the varieties in the 1941–42 test is regarded as quite significant in view of the earlier field tests and the equal, uniform exposure of all varieties to natural infection by the yellow dwarf virus in the randomized arrangement in which the varieties were replicated in this test. Sebago, Red Warba, and Russet Burbank again showed a definite, high degree of field resistance to the yellow dwarf virus.

The season averages of secondary disease and nonemergence are presented in table 1. Measurable differences in varietal reaction to

the yellow dwarf virus are definitely apparent. Russet Rural, Green Mountain, and Triumph are distinctly the most susceptible. Sebago, Warba, and Russet Burbank show a definite field resistance, and Rural New Yorker, Katahdin, Chippewa, and Irish Cobbler are intermediate in their resistance. In less exhaustive tests White Blossom Cobbler, Toanco 4, Mesaba, and Earlaine show field susceptibility, and Columbia Russet, Pioneer Rural, Pontiac, Houma, One Hundred-Day Cobbler, and Sequoia are additional intermediate resistant varieties.

In addition to the tests in 1940 and 1942, a commercial field planting conducted in the Almond area with the extra tubers of the Sebago seed stock that had been exposed to yellow dwarf infection in the 1939 adaptation planting showed little or no increase of the virus over a period of 3 years. When the Sebago tubers not used in the test planting in 1940 were increased, the percentage of disease was the same (6 percent) as in the 1940 test plot. In a 1941 planting of this same Sebago seed stock, only 8.5 percent of the plants showed secondary yellow dwarf late in the season. In a planting of the same Sebago seed stock again in 1942, only 5.5 percent of secondary yellow dwarf was observed, showing no increase over the 1940 and 1941 plantings. The high degree of resistance of the late-maturing Sebago variety under field conditions in the portion of central Wisconsin where yellow dwarf is very destructive is clearly evident.

CLOVER LEAFHOPPER POPULATION

No insect vector counts were made during the seasons of 1937 and 1939. In the experimental test planting in 1941, an increase in the clover leafhopper population was observed during the early season and counts were taken on all of the test varieties during mid-July and again in late August. A total of 25 sweeps was made on each variety in each of the replicates. The counts (table 2) show very little difference in population between the varieties under test. It is important to note in this connection that between each variety block, 4 rows of Russet Rural acted as buffers so that in no case were the test rows contiguous. Allen and Rieman (*1*), reporting on insects found on the Russet Rural and Sebago varieties in central Wisconsin in 1941, found little difference in clover leafhopper population on these 2 varieties, which is in accord with the present findings. These 2 late-maturing

TABLE 2.—*Clover leafhopper population counts taken on potato varieties in the experimental yellow dwarf field planting at Almond, Wis., on July 17 and Aug. 28, 1941*

Variety	Clover leafhoppers counted in 100 sweeps on—		Variety	Clover leafhoppers counted in 100 sweeps on—	
	July 17	August 28		July 17	August 28
	Number	*Number*		*Number*	*Number*
Green Mountain	292	364	Russet Rural	353	319
Sequoia	269	349	Red Warba	307	312
Triumph	344	384	Katahdin	264	347
Pontiac	296	327	Irish cobbler	261	341
Chippewa	278	321	Rural New Yorker	271	297
Sebago	284	309	Russet Burbank	230	279

varieties which show about the same population of clover leafhoppers in net collections are entirely different in their reaction to the yellow dwarf virus. The low yellow dwarf incidence of such varieties as Russet Burbank, Warba, and Sebago does not seem to be due, therefore, to any avoidance of these varieties by the vector. If resistance is due to escape, as was thought to be possible in an earlier report (*11*), it must be explained on the basis of ineffective transmission of the virus by vectors even though they are present.

GREENHOUSE EXPERIMENTS

In a series of experiments set up under controlled conditions in the greenhouse during the early winter of 1940–41 to study clover leafhopper activity, transmission, and resistance, three late-maturing varieties were compared. Green Mountain and Russet Rural were included because of their known susceptibility to the yellow dwarf virus and Sebago was selected because it had shown a very high degree of resistance under field conditions in the 1939–40 tests. Greenhouse tuber-indexed, disease-free seed was used in the trials which were conducted at a temperature that fluctuated between 24° and 28° C., favorable to both the insect vector and the expression of yellow dwarf symptoms.

In each of 3 transmission tests, 20 viruliferous adult clover leafhoppers were transferred by means of a special sucking pipette to each of 5 caged test plants from different tuber seed pieces of each variety. White sand was used to cover the soil in each caged test pot to facilitate observation of vector activity and to aid in the removal of the vectors after the inoculation period. A like number of caged plants from corresponding tubers with nonviruliferous leafhoppers and without leafhoppers was also included.

During the 14-day feeding period, frequent observations were made to determine the clover leafhopper activity on tne respectiv evarieties as to feeding habits, location of feeding, and longevity on a specific variety. No differences in leafhopper activity on the three varieties under test could be noted and there was no marked decrease in the number of live leafhoppers after the feeding period on any one variety.

At the end of the inoculation period all the viruliferous and nonviruliferous leafhoppers were removed from the cages by the aspirator method and the plants were held for observation. Symptoms of primary yellow dwarf developed on all of the 15 Green Mountain and Russet Rural test plants on which viruliferous leafhoppers were caged; in some plants symptoms were slightly more pronounced than in others. In the Sebago, only 4 plants out of a total of 15 in the entire series developed typical primary yellow dwarf symptoms; 2 plants developed faint symptoms. On 9 plants no recognizable primary symptoms could be seen. In plants on which the nonviruliferous leafhoppers were caged no symptoms were observed. All caged plants without leafhoppers also remained healthy. Tubers produced by the inoculated and uninoculated test plants were stored after harvest. The occurrence of secondary yellow dwarf in the plants arising from these tubers is recorded in table 3. The 15 second-generation Green Mountain and Russet Rural plants all showed typical secondary yellow dwarf symptoms, whereas, as stated above, only 6 inoculated Sebago plants showed typical symptoms. None of the test plants to

which nonviruliferous clover leafhoppers had been added or the non-insect-caged controls developed secondary yellow dwarf symptoms.

The greenhouse experiments confirm in a general way the field findings of Sebago resistance, although the percentage of infected plants was much higher in the greenhouse than in the field. The results show that although the same number of leafhoppers feed actively on each plant, certain Sebago plants are much less likely to be infected than Green Mountain or Russet Rural.

TABLE 3.—*The occurrence of yellow dwarf in the progeny of Green Mountain, Russet Rural, and Sebago plants inoculated with viruliferous clover leaf-hoppers in the greenhouse* [1]

Variety	Number of test plants inoculated	Number in which the progeny showed typical secondary yellow dwarf symptoms	Number in which the progeny showed no yellow dwarf symptoms
Green Mountain	15	15	0
Russet Rural	15	15	0
Sebago	15	16	9

[1] All uninoculated plants remained healthy.

DISCUSSION

The standard potato varieties Russet Burbank, Red Warba, and Sebago tested in an area of central Wisconsin where the yellow dwarf virus commonly builds up rapidly, showed a reasonable constant degree of resistance under field conditions.

The differences in seasonal yellow dwarf infection are governed by environment and influenced directly by the number of insect vectors present. There is, however, no direct correlation between the length of growing season, i. e., time of maturity of a variety, and resistance to the yellow dwarf virus. The two most resistant varieties under test were in one case a very early maturing one (Warba) and in the other a very late maturing one (Sebago).

Net sweepings on the potato varieties under test showed very clearly that the insect vector was equally abundant on all varieties. This in part has been observed by others (*1*). The low incidence of disease in certain varieties after exposure to heavy virus infection in the field is not the result of avoidance of any variety by the vector, as was though to be the case in preliminary studies (*11*), but rather in exclusion of the virus in certain individuals in a clone.

Whereas individuals of a variety, being members of a clone, are essentially buds from a single original plant, inherent difference between individual plants in true resistance can hardly be expected, since some members of the clone once infected are as susceptible as those of any other variety. When Sebago was exposed to viruliferous clover leafhoppers in the green house the percentage of infected plants was higher than in either of the two field tests. This would seem to indicate that the insect vector, even though as abundant on plants of the resistant variety as on those of the susceptible, does not actually cause as many cases of infection on the former in the field, but that the incidence of infection can be increased under certain

conditions in the greenhouse. This does not prove, however, that it is a matter of escape. Rather it shows that the plants escape infection not because the insects avoid them but because the insects are not able to infect them as readily as they do those of susceptible varieties. Whatever resistance may consist of, it is effective at the threshold of infection rather than after the virus has become established.

The low percentage of nonemerging hills in the more resistant varieties (table 1) is of special interest. The "poor stand" phase of the potato yellow dwarf disease under the conditions of relatively high soil temperature that prevail in central Wisconsin has been recognized as an important factor in potato production (*11*). It should be pointed out in this connection that varieties showing a high degree of field resistance, as a rule show also a low percentage of nonemerging hills. Better stands were obtained with resistant varieties exposed to infection than with susceptible varieties. Of even greater significance is the fact that virus-infected tubers of such varieties as Russet Burbank, Warba, and Sebago do not show to any extent the characteristic tuber malformation or the diagnostic internal rusty brown flecking, both associated with yellow dwarf infection, that is commonly found in the more susceptible varieties. This is an important item in relation to table market quality, particularly in the late-maturing varieties.

The planting of a late-maturing variety resistant to the yellow dwarf virus would not only increase yield and quality of table stock produced in the central Wisconsin area but would greatly reduce the cost of production with the elimination of the yearly purchase of seed stocks from yellow-dwarf-free areas.

SUMMARY AND CONCLUSION

The yellow dwarf disease of potato is an important factor in production in certain sections of Wisconsin where the virus commonly builds up rapidly in spite of the increased yearly planting of disease-free seed.

Varieties Russet Burbank, Warba, and Sebago have shown a high degree of field resistance to the yellow dwarf virus under conditions of extensive spread of the virus. This is indicated both by a low percentage of visibly infected hills and of nonemerging hills. These varieties when infected also show little if any tuber malformation or internal necrotic flecking.

In the field, the clover leafhopper, which transmits the disease, was found to be as numerous on resistant as on susceptible varieties.

No difference was observed in clover leafhopper feeding habits or location of feeding on the varieties under greenhouse culture tests. The chances of infecting certain Sebago plants are very much less than those of infecting Green Mountain or Russet Rural.

High incidence of the disease can be most effectively reduced by the use of a vriety or varieties resistant to the virus.

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SYSTEMIC INVASION OF CABBAGE SEEDLINGS BY THE DOWNY MILDEW FUNGUS[1]

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INTRODUCTION

In southern Mississippi it is a common practice to sow cabbage (*Brassica oleracea* var. *capitata* L.) seed in coldframes in October and early November to produce plants for the early spring crop. Epidemics of cabbage mildew (*Peronospora parasitica* (Fries) Tul. frequently occur and cause heavy losses in such plantings when the seedlings are 2 to 3 weeks old. In the course of investigations into the origin of these epidemics it was found that while the oospores of the mildew organism were rare in the diseased tissue of stems and leaves they did form in the cotyledons (*8*).[3] Since it is a common practice to grow cabbage plants in seedbeds at close quarters and on the same sites from year to year, it was obvious that oversummering oospores might be the chief source of inoculum in the fall sowing of plant beds. It is the object of this paper to present data on the mode of infection of cabbage seedlings by *P. parasitica* and upon the subsequent development of the disease, particularly with reference to development of epidemics in cabbage plant beds in southern Mississippi. The work was conducted at the Truck Crops Branch Experiment Station at Crystal Springs, Miss.

MATERIAL AND METHODS

Cabbage seedlings were grown from surface-sterilized seed planted in sterile soil either in cotton-stoppered fruit jars or in open cans. The inoculum, consisting of a suspension of conidia, was applied to specific predetermined points on various parts of the plants 5 to 10 days after emergence. That portion of the plant to which the inoculum was applied was kept humid for 24 hours, after which the plants were placed near a south window in the laboratory. Where plants were grown in open cans humidity was low enough to prevent sporulation and thus preclude secondary infection. When stoppered fruit jars were used the experiment was terminated at the occurrence of first sporulation.

For microscopic study the plants were cleared in lactophenol, stained with cotton blue in lactophenol, and mounted whole.

[1] Received for publication May 8, 1944. Contribution from the Mississippi Agricultural Experiment Station. Paper No. 81, New Series.

[2] The writer wishes to acknowledge his indebtedness to Dr. J. A. Pinckard for helpful advice during the course of these studies and to Drs. J. C. Walker and C. W. Edgerton for assistance in the preparation of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 463.

EXPERIMENTAL RESULTS

INFECTION STUDIES WITH CONIDIA

In each of two trials, five groups of plants were grown in cotton-stoppered fruit jars and kept in darkness for 3 days after emergence, with the result that the hypocotyls were from 5 to 8 cm. long. They were inoculated by placing a drop of a conidial suspension at the base of the hypocotyl, and the first signs of sporulation appeared 6 or 7 days later. The plants were then taken up and segregated with reference to the position of sporulation on the plant. Approximately the same number of plants grown under similar conditions, but not inoculated, remained healthy. The results of these experiments are presented in table 1.

TABLE 1.—*Relation of hypocotyl inoculation to point at which the mildew fungus sporulated after infection*

Experiment No.	Group No.	Plants inoculated	Total plants sporulating	Plants sporulating on—		
				Hypocotyl only	Hypocotyl and cotyledons	Cotyledons only
		Number	*Number*	*Number*	*Number*	*Number*
1	1	23	7	5	2	0
	2	19	4	3	1	0
	3	20	8	4	4	0
	4	15	5	2	3	0
	5	22	7	4	3	0
2	1	12	6	4	2	0
	2	15	8	5	3	0
	3	14	8	7	1	0
	4	10	7	6	1	0
	5	18	13	9	4	0

Representative plants from each group were cleared, stained, and examined microscopically. Of those plants on which no signs of sporulation occurred 25 percent were shown to have been infected by the presence of mycelium in the hypocotyl tissues. Examination of plants bearing sporulation revealed that the mycelium had progressed upward through the hypocotyl, extending into the cotyledons in some cases.

Of the 40 infected plants studied in detail, only the hypocotyl was invaded in 6; the hypocotyl and 1 or both cotyledonary petioles in 6; the hypocotyl, 1 or both cotyledonary petioles, and the basal portion of one or both cotyledons invaded but with no sporulation in 18; all 3 regions were invaded and sporulation occurred on the cotyledons in 10; the lower portion of the hypocotyl had been invaded in all. At a point corresponding approximately to the point of inoculation, the cortical tissue of the hypocotyl was completely invaded by a network of branching hyphae. Upward from this point branching decreased until there remained a comparatively few straight large hyphae which ran intercellularly and parallel to the long axis of the plant. Where branches occurred they usually followed a path parallel to and only a few cells removed from the mother hypha. The hyphae were abundantly supplied with large, well-defined haustoria (figs. 1 and 2). Continuing upward through the cortical tissues of the hypocotyl these hyphae passed through the cotyledonary node into the cotyle-

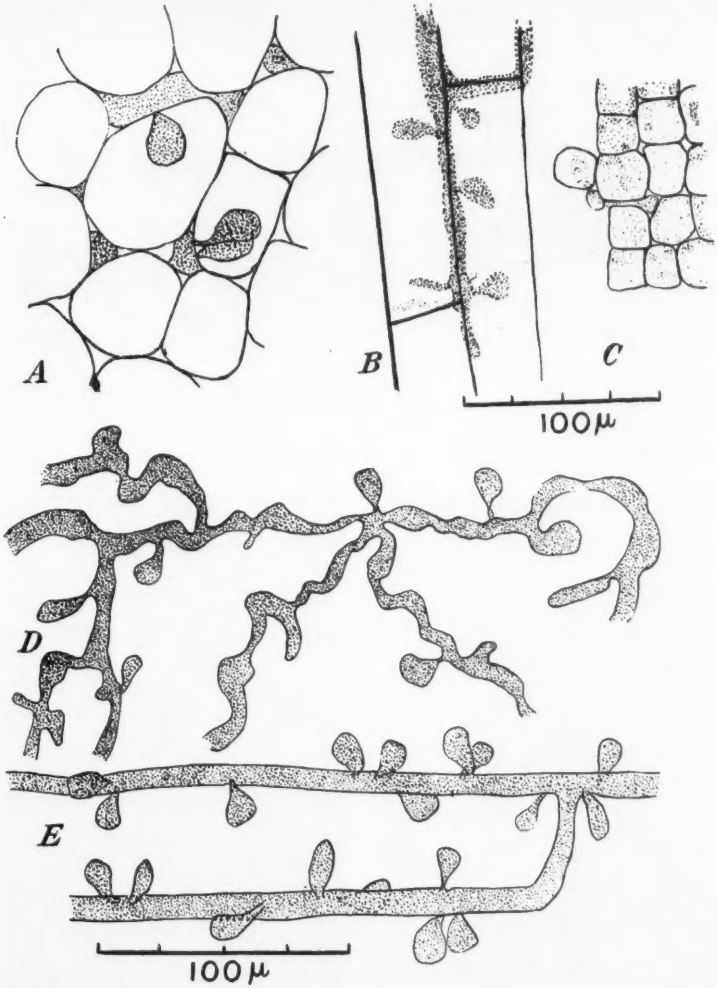

FIGURE 1.—Semidiagrammatic drawings of certain aspects of the pathological histology of cabbage seedlings systemically infected with *Peronospora parasitica* (drawings made with aid of a camera lucida). *A*, Transection of cortical tissues of hypocotyl of a 7-week-old cabbage seedling which survived systemic invasion by *Peronospora parasitica*, illustrating the intercellular mycelium and haustoria. *B*, Longitudinal view illustrating the characteristic branching of the systemic mycelium in the hypocotyl of an infected plant. *C*, Germination of a conidium and penetration of the germ tube between the epidermal cells of the hypocotyl of a cabbage seedling. *D*, Characteristic systemic mycelium as seen in cleared and stained whole mount of a cotyledon of a systemically infected cabbage seedling. *E*, Characteristic systemic mycelium as seen in a cleared and stained whole mount of the hypocotyl of a systemically infected cabbage seedling.

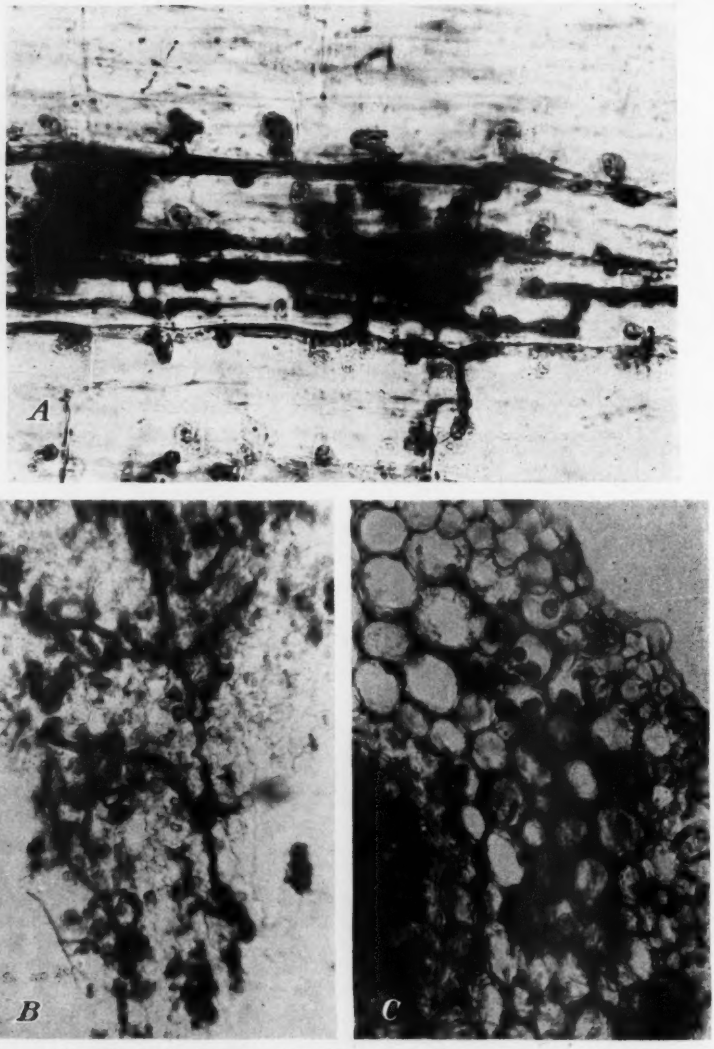

FIGURE 2.—Photomicrographs of cabbage seedlings systemically infected with *Peronospora parasitica:* Cleared and stained whole mount of a hypocotyl (*A*) and freehand tangential section of a cotyledon (*B*), showing characteristic systemic mycelium; transection of the hypocotyl of a 7-week-old seedling (*C*) which survived systemic invasion (note haustoria in the cortical cells).

donary petiole and finally into the lamina of the cotyledon. Tufts of conidiophores appeared at occasional points along the hypocotyl and cotyledonary petiole. In many instances in cleared and stained material single hyphae were traced from the base of the hypocotyl

through the intervening tissues into the cotyledon. At the base of the cotyledonary blade the long straight hyphae lost their identity and the uniform tubular hyphae changed into an irregular, knotty, much-branched mycelium with few well-defined haustoria (figs. 1 and 2). From masses of branching mycelium beneath the stomata conidiophores pushed through the stomata. Except for one questionable case, the mycelium never passed through the cotyledonary node into the next internode of true stem tissue.

In another trial seedlings growing in sterile soil in tin cans were inoculated with a suspension of conidia 5 days after emergence. The plants in each of the five cans were divided roughly into two groups by a piece of cardboard pressed down edgewise into the soil surface. The plants on one side were inoculated by placing a drop of the conidial suspension in a small depression of the soil at the base of the hypocotyl. The soil was then covered with moist absorbent cotton. The remaining plants were similarly treated, sterile water being substituted for the spore suspension. After 24 hours the absorbent cotton was removed and the plants were incubated in the dry atmosphere of the laboratory. Eight days after inoculation two of the five groups of plants were placed in a moist chamber. Five days later the remaining three groups were similarly treated. A record of the sporulation on these five groups of plants is presented in table 2. The controls in all cases remained healthy.

TABLE 2.—*Sporulation on plants inoculated with Peronospora parasitica at base of hypocotyl and placed in moist chambers 8 and 13 days after inoculation*

Group No.	Plants inoculated	Period from inoculation to placement in moist chamber	Plants showing sporulation in—			
			9 days	10 days	11 days	14 days
	Number	*Days*	*Number*	*Number*	*Number*	*Number*
1	7	8	6	0	0	
2	13	8	7	2	2	
3	8	13				6
4	10	13				5
5	11	13				8

After observations on sporulation had been made the plants were cleared, stained, and examined microscopically. In each case of plants on which sporulation developed the hypocotyl was invaded from the base upwards, and the mycelium extended into the cotyledons. These plants presented the same picture of pathological histology as described in the previous experiment. Without exception, whenever the cotyledons were invaded the hypocotyls were also affected, indicating that in all cases infection had occurred at the point of inoculation and not by the chance infection of the cotyledons by conidia. In no case was the mycelium found to have invaded the true stem tissue above the cotyledonary node.

An interesting observation was made on the effects of systemic invasion of the seedling tissues by the mildew fungus. At the time the last three groups were placed in the moist chamber, many of the plants showed collapse of the hypocotyl resembling the signs of damping-off although no other symptoms of mildew prevailed. The

cotyledons were shriveled, necrotic, and desiccated (fig. 3, *A*, *c–h*,). On the other hand, the plants of groups 1 and 2 (fig. 3, *B*, *c–e*) which had been placed in the moist chamber 5 days previously and before signs of injury were apparent, were all turgid. Aside from the mass of sporulation on the affected tissues, they appeared to be vigorous in spite of the disease. The importance of this observation in interpreting certain aspects of disease epidemics in the field will be discussed later.

In a third experiment 12-day-old cabbage seedling growing in sterile soil in tin cans were inoculated at a point approximately 2 cm. above the soil surface. This was accomplished by cutting slits in small corks, which were so placed that they held firmly around the hypocotyl leaving one side exposed. A drop of a suspension of conidia was applied to the exposed portion of the hypocotyl and covered with a bit of moist absorbent cotton. After 24 hours of incubation the cotton and corks were removed. Beginning 2 days after inoculation, 10 plants each day were severed at the soil line, cleared, and stained for microscopic examination. The controls all remained healthy.

As may be seen from the results in table 3, the invading mycelium rapidly progressed upward from the point of inoculation, finally reaching the cotyledons, after traversing an average of approximately 2 cm. of hypocotyl tissue in 5 to 9 days. As in previous experiments, no stem tissue above the cotyledonary node was invaded. The downward invasion of the hypocotyl tissues progressed more slowly and in only four cases did the mycelium reach the soil level, which was about 2 cm. below the point of inoculation. In other experiments in which the plants were taken up by the roots the mycelium was observed to progress as far as the point of origin of the uppermost secondary roots. During the last 3 days of the experiment, as in previous trials, definite signs of injury were apparent although no sporulation occurred since the plants were kept in the dry atmosphere of the laboratory.

The course of invasion of seedlings following infection of the cotyledons was followed in two experiments. In one of these a series of plants, the first true leaf of each of which was inoculated, was included for comparison. In these trials one cotyledon or the first true leaf was inoculated by placing a small drop of inoculum at about the center of the blade. Because of the waxy surface of the tissues a small drop of spore suspension could be made to adhere at the desired spot. A bit of moist absorbent cotton was placed at the base of each plant and a glass vial inverted over each seedling with the mouth pressed into the soil. After a 24-hour incubation period the vials and cotton were removed. The plants remained in the laboratory atmosphere throughout the experiment. No infection occurred in the controls.

When examined microscopically the major portion of each of the inoculated cotyledons was found to be completely invaded by the mildew mycelium. In most cases evidence of infection was apparent at the time the plants were taken up. The cotyledons were shriveled and necrotic while noninoculated cotyledons remained healthy. In some cases the mycelium had progressed from the lamina of the cotyledons into the cotyledonary petiole, and in some cases into the hypocotyl. The maximum extent of the invasion of the hypocotyl from the cotyledon was approximately 10 mm. When the first true

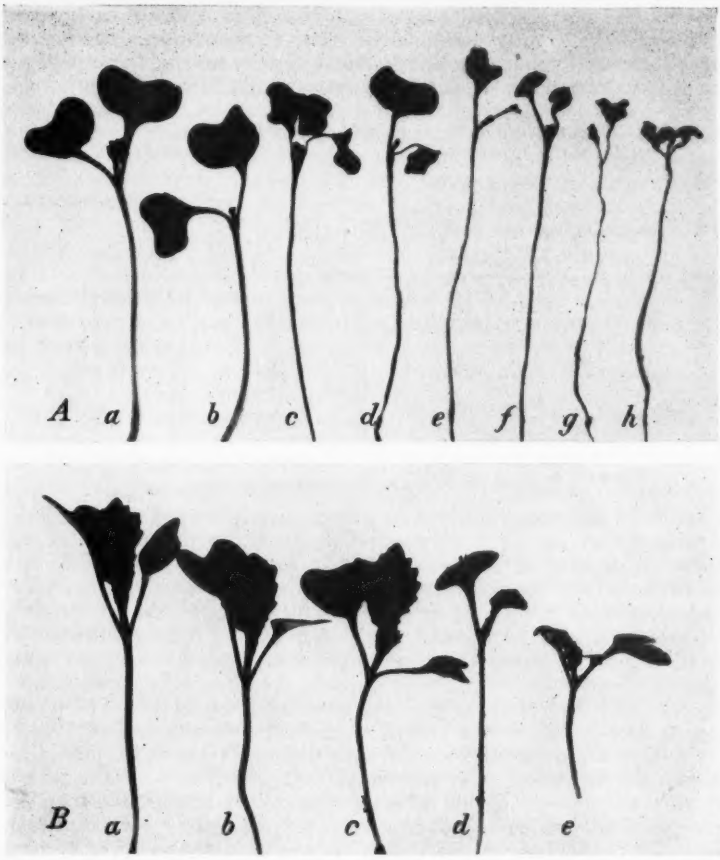

FIGURE 3.—Cabbage seedlings showing the effects of systemic invasion by *Peronospora parasitica*. *A*, Plants kept in a dry atmosphere: *a*, *b*, Healthy plants; *c*, *d*, *e*, *f*, *g*, *h*, infected plants showing different degrees of desiccation of the cotyledons and hypocotyls. *B*, Plants kept in a moist atmosphere: *a*, *b*, Healthy plants; *c*, *d*, *e*, infected plants showing tufts of conidiophores on the cotyledons and hypocotyls.

leaf was inoculated a necrotic spot 3 to 4 mm. in diameter developed at the point of inoculation. Microscopic examination disclosed that an area 3 to 4 mm. in diameter, roughly the area of necrosis, was invaded by the mycelium.

It has frequently been observed that in cabbage seedbeds many seedlings survive a mildew attack even when they have been infected while very young. Surviving plants from such a seedbed sown on November 5, 1942, were selected at the 7-leaf stage, when the hypocotyls were 3 to 5 mm. in diameter, and were sectioned and stained. In 9 of the 28 plants mycelium of the mildew fungus was present in the outer cortical tissue of the hypocotyl, but it had failed to pass

beyond the cotyledonary node into the stem tissue. It appears, therefore, that in plants which survive systemic invasion, the mycelium may remain in the cortical tissues of the hypocotyl for several weeks but does not spread systemically into the stem.

TABLE 3.—*The rate of upward and downward growth of the invading mycelium of Peronospora parasitica in the hypocotyl of cabbage seedlings*

Days after inoculation	Plants studied	Plants infected	Plants in which mycelium reached—		
			Cotyledonary node	Cotyledonary blade	Soil level
	Number	*Number*	*Number*	*Number*	*Number*
2	10	8	0	0	0
3	10	7	0	0	0
4	10	9	2	0	0
5	10	10	3	1	0
6	10	6	5	3	0
7	10	9	7	5	0
8	10	8	8	7	1
9	10	10	10	10	1
10	10	5	5	5	2

INFECTION BY SOIL-BORNE OOSPORES

Soil from a cabbage seedbed in which there had been an abundance of oospores in the cabbage seedling crop of the previous year was placed in each of 12 flats. The soil in 4 flats was disinfected with chloropicrin. Surface-sterilized seed was sown deeply on September 5 and the flats were kept in a coldframe covered with cheesecloth. Pairs of flats were segregated from each other in the coldframe by board walls. Beginning 5 days after emergence, the plants were watered frequently to provide suitable conditions for sporulation. Twelve days after planting the first sporulation appeared. The plants bearing sporulation were removed on each of 3 successive days. Since 3 to 4 days are required from the time of inoculation with conidia for sporulation to occur, it is presumed that few if any of the plants recorded as infected became infected from the conidia produced on the first plant to produce sporulation. The experiment was repeated, beginning on September 24. Infection was obtained in 11 of 16 flats of nonsterile soil, while in only 1 of the 8 flats containing sterile soil did any plants become infected. Examination of infected plants revealed that the mycelium invaded the hypocotyl and progressed systemically in the same manner as described for infection produced artificially. While the results do not furnish complete proof that oospores in the soil were the source of the primary inoculum, they are highly indicative that such was the case. It would appear that in seedbeds in Mississippi initial infection comes from oospores in the soil. Plants thus affected are invaded systemically and when sporulation occurs conidia are provided as secondary inoculum for further dissemination and infection.

DISCUSSION

The question of overwintering or oversummering of *Peronospora parasitica* has been discussed by a number of investigators (*2*, *4*, *7*, *9*, *10*, *11*), and wild hosts have been widely held to be responsible for the carrying over of inoculum (*2*, *p. 297–300*; *4*, *9*). Recent studies on host

specialization in *P. parasitica* (*6*) have shown that the form which attacks cabbage in this country is peculiar to *Brassica oleracea* and does not attack a wide range of cruciferous species. Thus the earlier belief that wild hosts are responsible for the carrying over of inoculum of cabbage mildew appears not to be well founded. That the cabbage mildew organism may overwinter in old cabbage heads and stumps has been pointed out (*10*, *11*). This means of oversummering is very unlikely in southern Mississippi since cabbage fields are plowed under about 4 months before the seed for the next crop is sown, in which time, by virtue of high temperatures and frequent rains, all traces of old cabbage refuse disappear. The possibility that summer collards (*B. oleracea* var. *viridis* L.) in home gardens may serve as a means of oversummering of the mildew organism is also questionable. The collard crop is usually planted in August and therefore is separated from the cabbage crop by at least 2 months. Further study should be made, however, before collards are completely discounted as a source of inoculum for cabbage mildew.

It appears that oospores serve as the most likely means of oversummering of the cabbage mildew organism. The occurrence of oospores of the cabbage organism in Europe has been reported (*1*). The fact that oospores have been found in abundance in the tissues of infected cabbage seedlings (*8*) and the infection studies herein reported support this belief.

Infection studies with *Peronospora parasitica* have been rather limited. That the tissues of leaf, cotyledon, hypocotyl, and stem are susceptible to infection by direct penetration of infective hyphae from germinating conidia has been demonstrated (*3*, *6*). Systemic development of the mildew mycelium in mature cabbage heads in storage (*10*), in the field (*11*), and in turnip roots (*7*) has been reported, but so far as the writer knows, this is the first report of systemic development of *P. parasitica* in the tissues of cabbage seedlings. The fact that only the hypocotyl and cotyledon appear to be susceptible to this type of invasion is of much interest and appears to be of considerable importance in the epidemiology of the disease.

In southern Mississippi, where cabbage seed for plant production for the early spring crop is sown from October 15 to November 10, severe epidemics of mildew frequently cause a loss of 50 percent or more of young plants. The extent of the damage varies with the age of the plants when attacked and with weather conditions. The greatest damage results when plants are infected before the first true leaf is fully developed and when the weather after the appearance of the disease consists of several moist, cool days followed by a prolonged period of dry, sunny weather. The earlier moist period provides ideal conditions for abundant sporulation and spread of infection from the original centers, as pointed out by Felton and Walker (*6*), while the dry, sunny period brings about rapid desiccation and necrosis of the infected hypocotyl tissues resulting in death of many seedlings. If the initial period of abundant sporulation and rapid spread of infection is followed by a prolonged period of quite cool, cloudy weather the survival of plants is considerably greater. Apparently under such conditions the fungus is retarded by the low temperature, as pointed out by Eddins (*5*), while the seedlings gradually outgrow the disease.

If the seedlings have reached the 3- to 4-leaf stage when the initial infection appears, the greatest damage results if a prolonged period of

cool, moist weather follows. Under such conditions the damage suffered is primarily one of growth retardation rather than actual killing of the plants. During dry periods such plants rapidly recover from the disease. The ultimate damage is thus determined by the duration of the moist, cool periods. If such periods are of long duration or occur with relatively short intervening dry periods the plants may be set back as much as 3 to 4 weeks. If the dry periods are prolonged, recovery is more rapid and damage is very slight.

The relation of high and low humidities and temperatures on the development of mildew on cabbage plants in the 5- or 6-leaf stage has recently been studied by Felton and Walker (*6*). In comparing the development of mildew on infected plants which were subjected to continuous high humidity, to alternating periods of high and low humidity, and to continuous low humidity, it was found that the plants under continuous low humidity outgrew the disease most rapidly, especially at the high temperatures. These results under controlled conditions agree with the observed effects under field conditions in Mississippi.

This observed difference between the behavior of mildew on very young seedlings and on older seedlings may be interpreted in the light of the experimental results reported herein. It was shown that in the case of very young seedlings the tissues became thoroughly infected from a single point of inoculation. Therefore, once a general infection was established in the seedbed, the continued production of inoculum diminished in importance. A warm, dry period following a general infection, therefore, did not greatly retard the development of the disease, but rather hastened its course in the infected hypocotyls and made them increasingly susceptible to desiccation during dry, sunny days. That infected tissues rapidly dry out and become necrotic under conditions of low humidity was pointed out by Felton and Walker (*6*). With older seedlings, in which individual infection points affect only a localized area of the leaf tissues, the ultimate severity of the disease is determined by the sum total of infection points. It is obvious therefore that, to insure a continued severe disease epidemic, conditions favorable for contidued sporulation and infection must be maintained. It has been pointed out that mildew lesions rapidly cease to sporulate when exposed to low humidity (*6*). Thus continued high humidity is a prerequisite to severe mildew development on these older plants.

The presence of the systemic mycelium in the hypocotyl of plants surviving the mildew attack presents an interesting subject for speculation in view of the reports (*10*, *11*) on systemic invasion of mature cabbage heads by the mildew fungus. Whether a connection can be shown between these two phases of systemic invasion or whether the systemic mycelium in the hypocotl is sloughed off with the cortex as secondary growth proceeds remains to be determined.

SUMMARY

The results of investigations into the epidemiology and parasitism of the mildew fungus (*Peronospora parasitica*) on cabbage seedlings are detailed.

Young cabbage seedlings became systemically invaded by the fungus after infection of the hypocotyl and cotyledonary tissues.

The systemic development of the mildew mycelium was confined to the hypocotyl and the cotyledons, and did not pass through the cotyledonary node to invade the true stem tissues. Invasion of the true leaves remained localized about the point of infection.

Oospores were indicated as the chief source of primary inoculum in the initiation of mildew epidemics in cabbage seedbeds in Mississippi.

The relation of these results to the interpretation of the development of epidemics of cabbage mildew in seedbeds in Mississippi is discussed.

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TRANSPIRATION RESPONSES OF PERSIAN WALNUTS AND FILBERTS SPRAYED WITH BORDEAUX MIXTURE[1]

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INTRODUCTION

The widespread use of bordeaux mixture for the control of many plant diseases has made it the subject of numerous investigations. Included among these are studies of its effect on the physiological behavior of the plant. Most of the earlier work on this subject has been adequately reviewed by Duggar and Bonns (*1*),[2] Miller (*4*), and Foster and Tatman (*2*).

The results of previous investigations are conflicting. A number of workers reported finding that the rate of transpiration increased after the application of bordeaux mixture to potted plants or detached leaves. Other workers, however, have found that bordeaux mixture decreases the rate of transpiration or has little or no effect. Still others have found that bordeaux mixture has a retarding effect on photosynthesis and respiration, but little or none on transpiration.

As bordeaux mixture has recently been found to be effective for the control of walnut blight (*5*), caused by *Xanthomonas juglandis* (Pierce) Dowson, and the bacterial blight of filbert (*7*), caused by *Xanthomonas corylina* P. W. Miller et al., it was deemed advisable to ascertain its effect on the transpiration of Persian walnuts (*Juglans regia* L.) and filberts (*Corylus avellana* L.).

MATERIALS AND METHODS

The experiments reported herein were conducted in the greenhouse at Oregon State College, Corvallis, Oreg., from 1939 to 1941, inclusive. Seedling Persian walnuts, the seed of which came from grafted trees of the Franquette variety, and seedling filberts from trees of the Barcelona variety were grown in cans. Coarse gravel to a depth of several inches was placed in the bottom of each of a number of watertight, lacquered, 1-gallon tin cans. An enriched soil composed of approximately equal parts of sand, well-rotted manure, and loam was put above the gravel. One seed of walnut or filbert, previously stratified in sand, was then planted in each can. When the seedling was sufficiently large for use, a rubber boot was placed about the base of the stem to prevent scalding of the tissues and the surface of the soil was covered with a mixture of melted paraffin and vaseline to reduce surface evaporation to a minimum. The soil was kept at the optimum moisture content by the addition of measured amounts of aerated tap water through a piece of glass tubing extending to the bottom of the

[1] Received for publication February 19, 1944. The investigation was carried on in cooperation with the Oregon Agricultural Experiment Station. Published as Technical Paper No. 422 of the Oregon Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 469.

can. Loss of water through the tube was prevented by placing a small piece of wax on its mouth. By means of this device loss of moisture was reduced without the production of anaerobic conditions in the soil, as the entrance of fresh air in small quantities was possible around the stem, between the side walls of the can and the paraffin cover, which invariably pulled away from the can, and in the aerated water. Air was also present in the spaces between the gravel in the bottom of the can, as was evidenced by its forcible expulsion through the glass tube when water was added. The addition of the fresh, aerated water at frequent intervals resulted in a repeated interchange of air, which prevented the accumulation of carbon dioxide and the consequent development of anaerobic conditions.

The plants were paired on the basis of similar leaf areas as determined by tracing the margins of the leaves on paper and measuring them with a planimeter. . All except the two basal leaves on each plant were removed. This is permissible since the lower leaves of both walnuts and filberts are structurally the same as the younger, terminal leaves; the only difference is that the old leaves are less active. After the plants were paired, the daily transpiration losses were obtained for each plant for a period ranging from 5 to 10 days to ascertain their pre-spray transpiration ratios. Light and heat conditions in the greenhouse during the experimental period were maintained in as nearly normal condition as possible. The individual plants were moved about systematically on the benches every day to minimize the effect of any possible variations in environment which might occur because of the positions occupied by the plants. Such variations as may have occurred were further minimized by repetitions of the experiments at different times during the growing season and over a period of years. After their pre-spray transpiration ratios had been established the upper and lower bottom surfaces of the leaves of one member of each pair were thoroughly sprayed with a hand sprayer. The formulas of bordeaux mixture used were 6–2–100, 6–6–100, 8–2–100, and 8–8–100; the 6–6–100 formula was used in about three-fourths of the experiments with walnut and the 8–8–100 formula in about half of those with filbert. Daily transpiration losses for the sprayed plant and for the corresponding untreated plant of the pair were then obtained for a subsequent period ranging from 5 to 21 days, and the ratio of the transpiration of the sprayed plant to that of the unsprayed was determined.

TRANSPIRATION RESPONSES TO BORDEAUX MIXTURE

The rate of transpiration after spraying with bordeaux mixture increased in 41 percent of the tests conducted with walnuts, totaling 124 pairs of plants, and decreased in 59 percent. In 54 percent of the tests conducted with filberts, totaling 59 pairs, transpiration increased and in 46 percent it decreased after the leaves were sprayed with bordeaux mixture. Representative results of the experiments are given in table 1. It is evident from an inspection of the data that the results are conflicting. The extent of the increase or decrease in the transpiration rate varied greatly in the different tests. It is worthy of note that, in those cases where the transpiration of the sprayed plants increased after they were sprayed, the acceleration occurred

TABLE 1.—*Representative transpiration ratios of Persian walnuts and filberts before and after being sprayed with bordeaux mixture, 1939–41*

Plant, bordeaux treatment, and pair No.[1]	Pre-spray ratios (to be sprayed: to remain unsprayed)	Post-spray ratios (sprayed: unsprayed)	Difference (pre-spray −post-spray ratio)[2]
Persian walnut:			
6–2–100:			
1	1.02	0.92	−0.10
2	1.01	.98	−.03
3	.50	.41	−.09
4	1.52	1.62	+.10
5	.73	.80	+.07
6	.60	.60	.00
7	.72	.70	−.02
8	.94	.70	−.24
9	.83	.95	+.12
10	.47	.38	−.09
6–6–100:			
11	1.04	.74	−.30
12	1.19	1.15	−.04
13	1.14	1.35	+.21
14	.95	.82	−.13
15	1.23	1.26	+.03
16	1.00	1.80	+.80
17	1.03	.63	−.40
18	.80	.93	+.13
19	.82	1.04	+.22
20	1.01	.97	−.04
Filbert:			
6–2–100:			
21	1.00	1.14	+.14
22	.81	.67	−.14
23	.91	1.18	+.27
24	.75	.59	−.16
25	.95	1.71	+.76
26	.96	1.03	+.07
27	.96	.90	−.06
28	.60	.64	+.04
29	1.01	1.19	+.18
30	1.29	1.51	+.22
Filbert—Con.			
6–6–100:			
31	0.94	1.05	+0.11
32	1.51	1.32	−.19
33	.93	.90	−.03
34	.71	1.04	+.33
35	1.25	.70	−.55
36	.93	1.13	+.20
8–2–100:			
37	1.32	1.61	+.29
38	1.62	1.52	−.10
39	1.75	.90	−.85
40	1.32	1.10	−.22
41	1.26	1.03	−.23
42	1.46	1.49	+.03
43	1.40	1.23	−.17
44	1.05	.90	−.15
45	1.39	1.13	−.26
46	.85	1.11	+.26
8–8–100:			
47	1.30	1.35	+.05
48	1.13	1.05	−.08
49	.90	.95	+.05
50	.47	.67	+.20
51	1.09	.95	−.14
52	.61	.55	−.06
53	.92	1.06	+.14
54	.96	.90	−.06
55	.47	.35	−.12
56	.99	1.21	+.22
57	.81	.81	.00

[1] Paired on the basis of similar leaf areas.
[2] Plus sign indicates an increase in transpiration; minus sign, a decrease.

immediately after spraying and then the transpiration of the sprayed plants decreased rapidly until the plant was transpiring at or close to the pre-spray rate.

From a hasty survey, it might be concluded that the wide variability in the data given in table 1 was due to poorly matched test plants. However, as previously stated (p. 466), the plants were first paired on the basis of leaf area, a basis for pairing used by a number of investigators (*2*, *4*). The transpiration responses of the paired plants for a 5- to 10-day period before spraying were then ascertained. Strange as it may seem, the transpiration rate of two plants having practically the same leaf area varied widely in many cases; this explains why the pre-spray ratios, as recorded in column 2 of table 1, varied so much.

The results of studies carried on by Wilson and Runnels (*8*), Horsfall and Suit (*3*), and others indicated that the higher the proportion of lime in bordeaux mixture the greater the increase in the transpiration rate. Wilson and Runnels (*8*) reported that a 6–6–100 bordeaux mixture increased the transpiration rate of sprayed coleus plants about twice as much as did a 24–6–100 bordeaux mixture.

Investigations carried on by the senior author (*6*) have shown that a highly alkaline bordeaux mixture causes considerably more damage to walnut foliage than does a low-lime or neutral bordeaux mixture.

It was, therefore, deemed advisable to determine the comparative effects of low- and high-lime bordeaux mixtures on the transpiration rates of walnuts and filberts. In table 2 are summarized the results of pertinent experiments.

TABLE 2.—*Comparison of transpiration responses of Persian walnuts and filberts to spraying with low- and high-lime bordeaux mixtures, 1939–41*

Plant and bordeaux formula	Number of pairs	Transpiration rate after spraying	
		Increased	Decreased
Persian walnut:		*Percent*	*Percent*
6–2–100	11	63.6	36.4
6–6–100	6	50.0	50.0
Filbert:	18	44.4	55.6
6–2–100	23	47.8	52.2
6–6–100			
8–2–100	21	47.6	52.4
8–8–100	62	50.0	50.0

It is evident from table 2 that the transpiration responses of both Persian walnuts and filberts after the application of a highly alkaline bordeaux mixture were not markedly different from those observed after the application of a low-lime bordeaux mixture. This may possibly be due to the use of mature leaves in these experiments. It is a known fact that in nature old leaves are much less active physiologically than younger ones.

DISCUSSION

The most distinctive feature of the data reported herein is their great variability. Just why the transpiration rate should increase after the application of bordeaux mixture in one experiment and decrease after its application in another carried on under the same or similar conditions is difficult to understand. It would normally be expected that within certain limits the plants of the same age and vigor, grown under the same environmental conditions, would respond similarly to any given treatment. In certain instances there were slight differences in the age of the leaves of the test plants, though the consistent use of the basal leaves in all tests should have minimized the chance of the differences in the activity level being mainly responsible for the variability of the results. Some of the variability could conceivably be due to differences in the number of stomata per leaf, though counts of them on representative leaves of comparable ages on different plants showed no marked differences in the number present. Since the plants used were seedlings, it is possible that the variability of the results may have been due to the existence of inherent, biological differences in the individual plants. There is the added possibility that the inconsistencies may be associated with the prevalence of structural or physiological differences between different groups of plants by virtue of differences in the fertility of various soil mixtures used in the different seasons.

The lack of a consistent response to applications of bordeaux mixture may possibly be associated with the use of older leaves in these experiments. It is quite possible that these mature leaves did not respond so readily to bordeaux mixture as younger, more active leaves might have done.

SUMMARY

The results of investigations on the transpiration response of Persian walnuts and filberts to spraying with bordeaux mixture are reported.

Under the conditions of these experiments no uniform effects on the transpiration rate of either Persian walnuts or filberts followed the application of bordeaux mixture. The response of both walnuts and filberts after being sprayed with a high-lime bordeaux mixture were not markedly different from those of plants sprayed with a low-lime bordeaux mixture.

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U. S. GOVERNMENT PRINTING OFFICE: 1945